VOLUME TWO

NUMBERS 1 & 2

INDIAN JOURNAL OF MYCOLOGICAL RESEARCH

(Official Organ of Mycological Society)

Edited by

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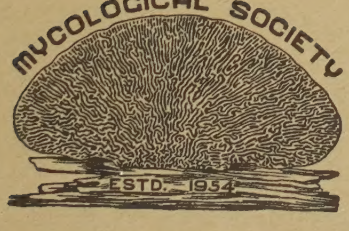

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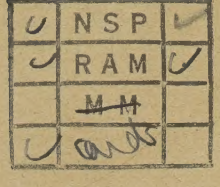

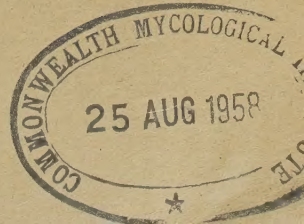

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INDIAN JOURNAL
OF
MYCOLOGICAL RESEARCH

(*Official Organ of Mycological Society*)

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Ind. J. mycol. Res., 2 (1 & 2), 1-28 (1956).

VARIATION IN *POLYSTICTUS XANTHOPUS* Fr.

By

SACHINDRANATH BANERJEE and HIMANI DEBI

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(With Plates I to IV and 4 Text-figures)

The present study has been undertaken to determine the degree of variation and the differences that exist between the variants in *Polystictus xanthopus* Fr. So far, three forms of the fructifications of the fungus have been recognised based only on the nature of the hymenial surface and they have been reported mainly from the hills of Assam. They usually grow on logs of *Shorea robusta* under identical environmental conditions.

It has been found that all the three forms of the fungus differ not only in the characters of the hymenial surface but also in the external morphology and microscopic anatomy of the sporophores, in growth characteristics on artificial and natural media, in oxidase reactions, in the rate of decaying sapwood of *Shorea robusta* and in the reaction to a toxic substance. Some of these characteristics have been found to be overlapping. The differences between the three forms in growth characteristics are both morphological, such as colour, topography and texture of the superficial mats, and physiological, as exemplified by the existence of antagonism between the uniting pairs of mycelia on artificial media, effects of variation in *p*H of a medium on growth, reactions to zinc chloride and the rate of growth. No hyphal fusions between the polysporous mycelia of the three forms have been observed.

Introduction

There are records (Stakman, 1929) of many species of fungi which exhibit physiologic forms. These forms differ greatly in their parasitic activity, growth characteristics under cultural conditions and also to some extent in morphological characters. Among Hymenomycetes, wide variation of this type within the same species has been noted only in a few species by Schmitz (1925) and Verrall (1934). In any comparative work it is, therefore, essential to find out the range of variation existing within the same species in the characters being studied. Considerable cultural work has already been done with wood-rotting fungi from India by Bose (1929, 1929a, 1930), Banerjee and Bakshi (1945), Bagchee and Bakshi (1950) and Bakshi and Bagchee (1950), but no work has been done on form-variation on any species in India so far. It is, therefore, desirable to study on a comparative basis the range of variation exhibited by a given species.

The present study has been undertaken to determine the degree of variation existing in *Polystictus xanthopus* Fr. This fungus has been selected, because Bose (1936) has reported form-variation within this species mainly on the characters of the hymenial surface. According to him "the hymenial surface of specimens of *Polystictus xanthopus* Fr.

at high altitude shows three different kinds of porous area, viz., some with typical very small pores, some with much bigger pores and some with hydnoid pores". He also concludes that they are variations of one and the same species. Such variations, however, are hardly found in the specimens collected from the plains. Bose's report has been based on materials from different localities of Cherrapunji (8000 ft.) and Lokra hills (8000-10,000 ft.) Assam, collected in 1929 and 1934 respectively.

So far as the authors are aware, very little work has been done on this fungus up to the present time. Although first named and described by Fries (Saccardo, 1888), it was subsequently re-described by various workers like Lloyd (1910), Bose (1918, 1919, 1936), Corner (1932) and others based on collections from different localities. Their descriptions were mainly based on external morphology of the fruit-bodies. In 1932, Corner not only described the characteristic form of *Polystictus xanthopus*, but also extended his researches on the hyphal anatomy of the fruit-body of the fungus. Further, its sexuality has been recently studied by Banerjee and Samadder (1957). Nothing more in particular, is known about the fungus on other aspects.

The scope of the present investigation is to determine how much this species varies with regard to external form and anatomy of the fruit-bodies together with general cultural characteristics on artificial media including the rate of growth, habit of growth, colour-variation and hyphal characteristics of the mycelium. Attempts have also been made to find out the nature of antagonism and compatibility that exist between the mycelia of different forms, their rate of decaying wood and also their response to a toxic substance of different concentrations.

Geographical Distribution

Polystictus xanthopus Fr., a common saprophyte, has been known for more than a century in the tropics of the old world. In India, it is known to occur on logs and fallen branches of *Shorea robusta* Gaertn.f. and on *Cedrela toona* Roxb. (Banerjee 1947; *List of common names of Indian Plant Diseases, I. C. A. R.*, 1950) causing white spongy rot. Lloyd (1910) reports that the home of this plant is Africa and probably it was first published by Ehrenberg as *Polyporous katui* which was subsequently re-named as *Polystictus xanthopus* by Fries. The African collections run to the mesopodial types mainly. In tropical America they are very rare. There are records of this species from America in Saccardo (1888), but according to Lloyd (1921), they are erroneous, as in his wide experience he did not receive a single specimen from this country. More recently, however, Killermann (1928) has again stated it to be pantropical. Petch (1916), mentions that it generally occurs in low countries. According to Corner (1932), besides these, there are collections in Singapore herbarium from many of the low lying parts of Malaya and specimens from high altitudes, such as from Camerons highlands in Pahang (6000 ft.), have also been found. In Fraser's hill, Pahang, (4000 ft.) luxuriant growth of this fungus has been reported. It has also been reported from Negri Sembilan

on Gumong Angsi (2700 ft.). Therefore, it can be stated that *Polystictus xanthopus* seems to be generally distributed throughout the Peninsula and is independent of altitudinal variations. Saccardo (1888) reports it to be commonly found in the tropics, Sierra Liona (Africa), India, Java, Guyana, Brazil, Peru, Pegu, Sumatra, Cocincina (S. America), Australia, North America and Islands of the Pacific Ocean. It has also been reported from various places in India by Bose (1919, 1936) and others (Butler and Bisby, 1931).

Materials

The fructifications used for the study were collected from Cherrapunji, Assam, in 1953, in the month of September. The situation was rather cool, very damp, somewhat shady and provided a favourable condition for the growth of the fungus. They were found to grow luxuriantly on posts and logs of *Shorea robusta*. Several collections of the fungus from this locality were made from which three morphologically different types were carefully selected. The materials when brought into the laboratory, were revived by placing the fructifications with their upper surface downwards on moist cotton wool in several Petri-dishes and from these spores were collected in sterile agar plates. From these spores initial polysporous cultures were made by transferring loopfulls of spores aseptically to 2.5% *malt-agar* slants. These were then incubated at 30°C. Within a week good mycelial growth was obtained in all the tubes and these were finally kept as stock-cultures for subsequent study and comparison. Microscopic examination of all the cultures showed the presence of much branched, hyaline, septate hyphae with abundant clamp-connections.

Morphology of the Sporophores

A. *External features*

It has been previously stated that there exists considerable variation in external morhpology of the fruit-bodies of *Polystictus xanthopus* which first attracted the attention of Bose in the year 1936. He recognises three different types of fructifications based mainly on the character of the hymenial surface. After careful study of the external features of all the materials three distinct types of fruit-bodies have been seggregated as follows :

Type–I. Fructifications with long and narrow excentric stipe, very thin, more or less obliquely infundibuliform and with very minute, regular pore-mouths ;

Type–II. Fructifications small, with somewhat stout and thick excentric stipe, more or less obliquely saucer-shaped, with a central depression and with minute, regular and slightly larger pore-mouths ;

Type–III. Fructifications thin, always sessile, bracket-shaped and with angular to 'Irpecoid' or 'Hydnoid' pore-mouths.

Forms with intergrading characters have also been noticed in many specimens, but these cannot be strictly placed in either of the above-mentioned three distinct categories. The external features of the types recognized by the writers have been fully described below. In all cases the colour of the fruit-bodies has been expressed according to Ridgway (1912).

Type–I (Plate I, figs. 1 and 2)

Fructifications : Stipitate. *Stipe :* Usually excentric, sometimes central but never lateral, more or less cylindrical, arising from a small disc-like concolorous base, gradually expanding upwards into the pileus but sharply delimited from it, smooth, Ochraceous-Buff to Ochraceous-Orange in colour, often with Ferruginous spots, somewhat stiff on drying, dimension about 10–25×1–3 mm. *Pileus :* Infundibuliform, usually obliquely so; coriaceous, becoming stiff on drying; sometimes laterally confluent; usually about 7–9 cm. or more in diameter; margin very thin, entire, even, becoming lobed in larger, specimens, involute on drying; white but becoming Cinnamon-Buff in dry specimens. *Upper surface :* Glabrous, shining; concentrically zoned; somewhat stiff; rugulose due to longitudinal wrinkles; colour showing various shades of Cinnamon-Buff, Orange-Cinnamon, Vinaceous Tawny; Garnet Brown and Maroon in regular concentric bands of variable thickness, Cinnamon-Buff colour being predominant in some specimens. *Context :* Very thin, less than 0.5 mm. in thickness; white; coriaceous. *Hymenial surface :* Smooth, somewhat longitudinally wrinkled in dry specimens; dull white in colour but becoming Pinkish-Buff when old; pore-mouths minute, regular, more or less circular, ending abruptly near the margin forming a sterile zone, about 1 mm. wide; pore-tubes very short, forming a distinct layer, not separable from the substance of the context.

Type–II (Plate I, figs. 3 and 4)

Fructifications : Stipitate. *Stipe :* Always excentric, never central or lateral; usually short cylindrical, sometimes elongated, dilating upwards into the pileus but sharply delimited from it, arising from a more or less concolourous discoid base; Ochraceous-Orange or Antimony Yellow in colour, sometimes with Ferruginous spots; smooth and glabrous, sometimes rugulose; somewhat stiff and corky on drying; dimension about 5–7 (12)×5–8 mm. *Pileus :* Always shallow, saucer-shaped but oblique; coriceous, becoming stiff on drying; sometimes laterally confluent; about 3.5–7 cm. wide and 2.5–4 cm. deep. Margin thin, usually entire, even, sometimes slightly wavy but never lobed, not involute on drying, at first white, but turning Pinkish-Buff with age. *Upper surface :* Glabrous; shining with numerous narrow to broad concentric zones; somewhat rugulose due to longitudinal wrinkles; colour showing various shades of Garnet Brown, Maroon and Claret Brown throughout, a broad band of Maroon colour invariably present just behind a narrow Pinkish-Buff margin; a prominent ride around the central depression almost invariably present. *Context :* Somewhat thick, less than 1 mm. in thickness; white; coriaceous. *Hymenial surface :* Usually smooth, sometimes with wide longitudinal

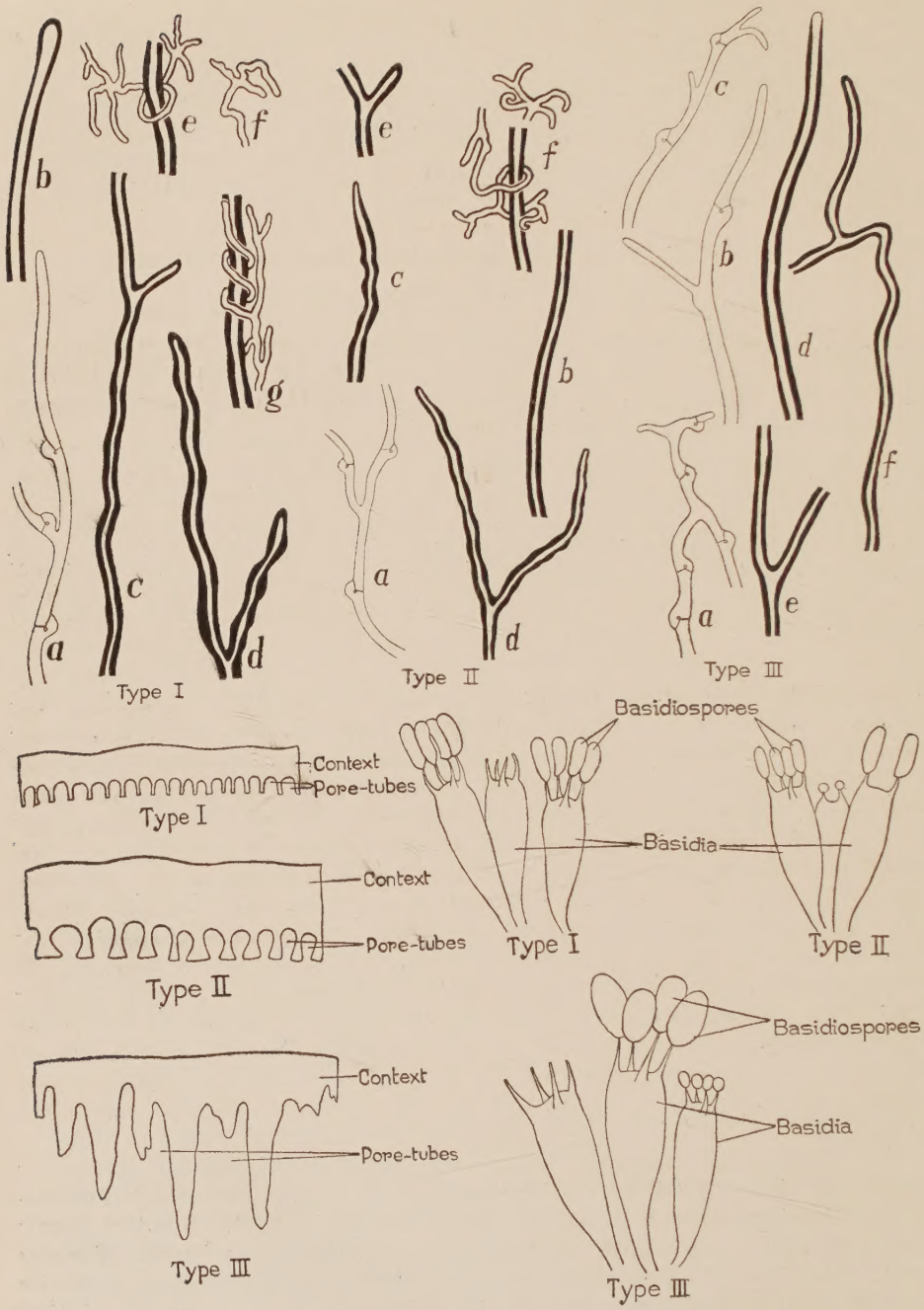
ridges and furrows; colour not uniform, a broad central light Wood Brown zone with Light Buff to Pinkish-Buff coloured areas towards the margin and the stipe; pore-mouths minute, regular, more or less circular throughout but delimited towards the periphery by a distinct narrow zone, about 1 mm. wide and consisting of somewhat angular pores; this narrow zone abruptly ends in a broad (2–3 mm. wide) sterile edge; pore-tubes comparatively large; tubes forming a distinct layer and not separable from the substance of the context.

Type–III (Plate I, figs. 5 and 6)

Fructifications : Bracket-shaped (reflexed), and sessile; usually attached to the substratum by a comparatively narrow base or the basal portion near the point of attachment with the substratum is slightly elongated resembling a stalk; thin; flabelliform, in the form of an arc of a circle; sometimes imbricate; coriaceous when fresh, becoming somewhat stiff on drying; about 3–7 cm. across and 2.5–4 cm. deep. Margin very thin, uneven, slightly lobed, not involute on drying; whitish at first but turning Pinkish-Buff to Warm Buff in dry specimens. *Upper surface:* Glabrous; somewhat shining; stiff; with numerous longitudinal wrinkles diverging from the base outwards and rendering the surface somewhat rugulose; faintly zoned, zones usually narrow but one or two zones usually very prominent and raised; colour Maroon and Garnet Brown throughout excepting the margin. *Context :* Very thin, less than 0.4 mm. in thickness, white, coriaceous. *Hymenial surface :* Rough and 'Irpecoid', somewhat folded towards the margin in bigger specimens, sometimes with one or two faint zones; colour Warm Buff towards the margin, other portions Antimony Yellow but changing to Cinnamon-Brown on drying; pore-mouths large, regular, and somewhat angular towards the margin, in older portions becoming teeth-like (Irpecoid) due to breaking down and also subsequent growth of certain portions of some of the dissepiments in between them, sometimes forming somewhat continuous cavities interrupted internally by short partition walls; a uniform sterile zone at the margin present, not raised as in the other two types; pore-tubes comparatively long, forming a distinct layer and not separable from the substance of the context.

B. *Microscopic Anatomy* (Text-fig. 1)

The microscopic anatomy of the fruit-bodies of *Polystictus xanthopus* was studied from free-hand sections mostly cut from pieces of dried sporophores following the method described by Overholts (1929). For temporary mounts sections were stained in lactophenol-cotton-blue with satisfactory result. For hyphal constituents of the fruit-body, thin sections from its different parts were at first treated with a few drops of 10% KOH solution on a slide and washed with water. Small groups of hyphae were then teased from these sections in warm lactophenol and examined without staining. Staining of the hyphae was considered unnecessary, because when the substage diaphragm and the condenser of the microscope were



Text-fig. 1. Anatomical features of the three types of fructications of *Polystictus xanthopus*. (Hyphal systems and basidia & basidiospores. × 700)
(For explanation, vide text)

slightly regulated, visibility of the hyphae was decidedly increased. These teased materials were also mounted in Erythrosine-glycerine-agar jelly for making permanent preparations. Measurements regarding the width of the hyphae and other elements or structures of any given specimen were based entirely, according to the suggestion given by Bisby (1945). Ten to twenty routine examinations and measurements of carefully selected elements to include the range of sizes were found to be sufficient for this purpose. The nomenclature adopted by Corner (1932) in describing different types of hyphae of the fruit-bodies have been followed.

(i) *Thickness of the context.* The thickness of the context varies greatly in the three types, maximum being in Type–II and minimum in Type–III. The thickness of the context in Type–I is intermediate between the two and varies from 300-420μ, that of Type–II and Type–III ranging from 600–870μ and 120–330μ respectively.

(ii) *Length of the pore-tubes.* The pore-tubes, with regard to length, vary greatly and do not form a well distributed series. The pore-tubes in Type–II are almost double in length than those in Type–I, but between Type–II and Type–III there is a wide gap. The length of the pore-tubes in Type–I varies from 80–140μ and that in Type–II 170–230μ. Maximum length of the pore-tubes has been attained in Type–III which varies from 660–1950μ.

(iii) *Thickness of the dissepiments.* As regards the thickness of the dissepiments it is also different in the three types. In Type–I it is narrow, more or less uniformly thick from base to apex of the pore-tubes and about 4–8μ in thickness. In Type–II the dissepiments appear as biconcave columns in longitudinal section, dilating towards the pore-mouths and the bases of the pore-tubes, and finally merging into the context. Its thickness varies between 8-12μ in the middle and about 16–20μ at either ends. In Type–III, the dissepiments appear as more or less conical structures in longitudinal section being widened towards the context and more or less tapering towards the pore-mouths. Its thickness varies from 12–14μ towards the apex and 44–48μ near the base.

(iv) *Diameter of the pore-tubes.* The diameter about the middle of the pore-tubes does not vary much in Type–I and Type II, being 60–110μ in the former and 60–120μ in the latter, but in Type–III it is very much widened and ranges from 150–300μ.

(v) *Length and width of basidia and basidiospores.* The shape and size of the basidia and basidiospores in Types I and II agree more or less closely with each other. The basidia in Type-III appear to be larger although the basidiospores do not vary much with those in other two types so far as their measurements are concerned. Table 1 will furnish the relevant data.

Table 1. *Basidia and basidiospores of* Polystictus xanthopus

	Type I	Type II	Type III
Basidia	Broadly clavate, tetra-sterigmatic and quadri-sporous; dimension about 12.2–13.5×3μ.	Broadly clavate, mostly di-sterigmatic and bi-sporous, some tetra-sterigmatic and quadri-sporous; dimension about 12—13×3—3.5μ.	Clavate, elongated, tetra-sterigmatic with long sterigmata and quadri-sporous; dimension about 18—19×5.5—6μ.
Basidiospores	Oval to cylindrical, sometimes slightly curved; hyaline, thin-walled, smooth; dimension about 5–5.5×2–2.2μ.	Shape and structure like those in Type–I; dimension about 5.4×1.5–2μ.	Mostly oval to sub-cylindric; hyaline, thin-walled, smooth; dimension about 6.4–7.5×3μ.

(vi) *Hyphal characteristics.* In hyphal composition of the fruit-bodies, there appear to exist two distinct groups. Fructifications of Types I and II together form one group in which all the four types of hyphae, viz., *generative*, *skeletal*, *mediate* and *binding*, as defined by Corner (1932), are present. Fructification of Type–III alone forms the other group in which there is a tendency towards the elimination of binding hyphae. The hyphal composition of the three types of fruit-bodies has been summarized in Table 2.

Table 2. *Hyphal system in the three types of fruit-bodies of* Polystictus xanthopus

Generative hyphae	Frequent; usually thin-walled, sometimes the walls become slightly thick but with uniform linear lumen; branched extensively or sparingly; longitudinal or interwoven; hyaline; about 1.5–3 (4) μ wide; usually nodose septate and having a simple clamp-connexion at each septum; with abundant protoplasmic contents, in the older portions contents becoming scanty. (Text-fig. 1, a)	Frequent; characters same as those in Type–I; about 2–6μ in diameter. (Text-fig. 1, a)	Very abundant; profusely branched and interwoven; width varying from 2–3 (4) μ; full of granular contents; nodose septate; branches often becoming narrow. coralloid, nodulose or spiculiferous; general characteristics like those in Type–I. (Text-fig. 1, a–c)

	Type I	Type II	Type III
Skeletal hyphae	Most abundant; thick-walled; straight or slightly flexuous; unbranched; more or less longitudinal; without clamp-connexions; hyaline to pale yellow in colour; about 2–6μ wide; wall thickness very variable, in some cases lumen almost obliterated in mature parts, sometimes uniformly thick throughout; apices mostly thin-walled, usually aseptate, but in a few occasional septa may be present. (Text-fig. 1, b)	Most abundant; characteristics more or less similar to those in Type–I; width of the hyphae 4–6μ. (Text-fig. 1, b–c)	Very abundant ; mostly flexuous but sometimes straight; usually interwoven due to flexuous nature; hyaline to yellowish brown in mass; about 5–6μ wide; other characters similar to those in Type–I. (Text-fig. 1, d)
Mediate hyphae	Frequent, more or less uniformly thick-walled, with lumen linear; usually aseptate, a few occasional septa may be present; pale yellow in colour about 1.5–3.5μ wide; straight or slightly flexuous; somewhat inter woven; scanty granular contents may be present; apices thin-walled with granular contents. (Text-fig. 1, c–d)	Present but less frequent; characters similar to those in Type–I ; about 2–4.5μ wide. (Text-fig. 1, d–e)	Present but comparatively very few; about 2–4μ wide; characters similar to those in Type–I. (Text-fig. 1, e–f)
Binding hyphae	Frequent; thick-walled; much branched; often intertwining or twisted, usually coiling around skeletal and mediate-hyphae, sometimes coralloid, nodulose or spiculiferous; without clamp-connexions; mostly hyaline to pale yellow coloured; about 1–2.5 (3)μ wide; wall thickness variable with lumen linear or obliterated in older parts; apices thin-walled with few granular contents. (Text-fig. 1, e–g)	Frequent; characters more or less similar to those in Type-I; width varies between1.5–2.5(3)μ. (Text-fig. 1, f)	Not in evidence ; structures appearing as binding hyphae are in reality much branched generative hyphae.

Fungus in Culture

A. *Cultural Characteristics*

(i) On agar medium

So far as the authors are aware, nothing is known about the cultural characteristics of *Polystictus xanthopus*. For studying the cultural characteristics of the three forms recognized by the writers, polysporous mycelia of all the types were grown separately on *potato-dextrose-agar* in Petri-dishes (90 mm. in diameter). The medium was prepared, according to the recipe of Fritz (1923). The cultures were grown in complete darkness under ordinary conditions of temperature (28°–30°C.) of the laboratory. In order to maintain the size of the inocula as constant as possible, small discs (5 mm. in diameter) of agar with the polysporous mycelium of each type were cut out from the periphery of the advancing mycelium by means of a sterile cork-borer from 7-days-old culture. Each disc was then placed with upside downwards upon the medium at the centre of the Petri-dish containing approximately about 30 c.cm. of the medium. The *p*H value of the medium after sterilization was found to be 5.2. Observations were based on an average of four Petri-dish cultures of each type. The terms used to describe the texture of the mat are those proposed by Nobles (1948) and the colour exhibited by the mycelium has been expressed, according to Ridgway (1912). The cultural characteristics of the three types have been described below separately.

Type–I (Plate II, fig. 7)

(i) *Habit of growth.* In all cases, growth started with much rapidity and within 24 hours after inoculation, formed a thin, cottony mycelium over the surface of the inoculum. Within the next 24 to 36 hours, growth appeared over the agar medium forming an appressed mycelium which later tended to become somewhat raised and cottony towards the periphery, while the mycelium over the inoculum turned somewhat felty. As the growth advanced, the superficial mat gradually condensed and in about six days after inoculation it was differentiated into an outer raised felty zone surrounding a central appressed area. On the tenth day the central zone was somewhat lacunose and the peripheral zone became slightly raised and felty to farinaceous. No further change in the habit of growth was, however, noticed even in cultures 15 to 20-days-old, excepting the mycelium appeared to be more compact. No change in colour of the medium was noticed in any case.

(ii) *Colour.* The colour of the mycelium was white throughout during the early period of growth, but from the eighth day pigmentation of the mycelium started. At first patches of Light Vinaceous appeared only in the central lacunose zone. A gradual change of colour was, however, noted from the ninth day onwards with the age of the culture and the range of colour included Vinaceous to Drab towards the centre and

Pinkish-Buff with Apricot Buff spots here and there towards the periphery.

(iii) *Rate of growth.* The rate of growth was more or less uniform from the very beginning and increased about 5 mm. daily in radial direction.

Type–II (Plate II, fig. 8)

(i) *Habit of growth.* Growth started with moderate rapidity forming thin mycelium over the inoculum within 24 hours after inoculation. On the following day it turned somewhat patchy felty and became surrounded by a thin, narrow zone of appressed mycelium over the agar medium. On the fourth day the major portion of the superficial mat became uniformly smooth but tended to become somewhat raised and felty towards the margin and with a narrow appressed zone of advance. Within a couple of days a somewhat rugulose area appeared in the central region of the mat which soon became separated from the inoculum by a slightly raised circular felty area. Towards the periphery of the mat, the texture remained uniform. Between the periphery and the rugulose region, the mat became slightly depressed and assumed Chamoise texture in culture, about 10-days-old. No change in colour of the agar medium was, however, noticed.

(ii) *Colour.* At the onset, the mycelium was pure white throughout but tinting of the mat started on the sixth day after inoculation. The central area of the mat at first became coloured and the range included Pinkish Cinnamon and Pinkish-Buff. Further change in colour was, however, not noticed excepting in that certain portions of the coloured area became more intense.

(iii) *Rate of growth.* To start with the growth was rather slow but within three to four days after inoculation it became more rapid and remained more or less uniform throughout in all cases. The average daily increment of growth was 5.5 mm. in radial direction.

Type–III (Plate II, fig. 9)

(i) *Habit of growth.* At first the growth was rather slow to start with and within 24 hours after inoculation practically no growth was observed over the inoculum. After 32 to 34 hours, thin, white sub-felty growth could be recognized only over the inoculum which within the next 12 to 16 hours descended over the medium forming a narrow circular zone of appressed and sodden mycelium. As the growth advanced, the superficial mycelium gradually became raised, smooth and felty forming a zone towards the periphery. On the tenth day the superficial mat became smooth and felty throughout, but with two to three raised concentric zones about the middle portion of the mat. No further change in texture was, however, noticed during later stages of development of the mycelium excepting in that it became more or less tightly interwoven. There was no discolouration of the medium during this period.

(ii) *Colour.* At the beginning the colour of the superficial mat was white throughout. On the sixth day of inoculation slight tinting of the

mycelium started at the periphery in the form of a circular zone of Pinkish-Buff while other portion of the mat remained white. With age, pigmentation gradually extended towards the centre showing lighter shades on the tenth day. Finally, the colouration of the mat deepened and showed gradual shades of Saccardo's Umber, Twany-Olive and Pinkish-Buff from the periphery to the centre.

(iii) *Rate of growth.* Although the growth started slowly, the mycelium soon became active and subsequently grew more or less at uniform rate. The daily increment of growth was found to be 4.5 mm. in radial direction.

In Table 3 a comparative account of the cultural characteristics of the mycelia of the three types of *Polystictus xanthopus* on the tenth day on *potato-dextrose-agar* has been summarized.

Table 3. *Comparative cultural characteristics of the mycelia of the three types of* Polystictus xanthopus *on the tenth day of inoculation on potato-dextrose-agar.*

	Type–I	Type–II	Type–III
Habit of Growth	Growth slightly raised, felty to farinaceous towards the periphery, surrounding a central lacunose zone.	Peripheral region somewhat raised and uniformly felty; behind it a slightly depressed Chamoise zone surrounding a central somewhat rugulose area.	Smooth and felty throughout but with two to three raised concentric zones about the middle portion of the mat.
Colour	Light Vinaceous towards the centre and a few Pinkish-Buff spots here and there towards the white peripheral mat.	Central portion of the mat showing shades of Pinksih Cinnamon and Pale Pinkish-Buff; other portions white.	Pinkish-Buff peripheral zone with lighter shades extending from the periphery towards the white central region.
Daily increment of growth	Uniform; 5 mm. in radial direction.	Uniform; 5.5 mm. in radial direction.	Uniform; 4.5 mm. in radial direction.

(ii) On wood-blocks (Plate II, figs. 10-12)

Growth characteristics of the superficial mycelia on sapwood blocks of Sāl were also studied (Plate II, figs. 10–12). For this purpose, several Kolle flasks, each containing sterile 2.5% *malt-agar* slants, were inoculated with polysporous mycelia of the three types of *Polystictus xanthopus* separately and after 10-days-growth, several water-soaked sapwood blocks were exposed to them. These flasks were then kept in complete darkness and room temperature (25°–30°C) of the laboratory.

After three to four days, almost all the blocks were attacked and partly covered by the mycelia, but the rate of growth in case of Type-III appeared to be rather slow. The mycelium in each case was white throughout at the beginning. On the seventh day after exposure of the wood-blocks, very luxurient growth of the mycelium of Type–II was observed and the texture was Chamoise like in appearance. In case of Type–I, the free faces of the wood-blocks were covered with densely felty to cottony mycelium, which later became more active and uniformly compact. The growth of the mycelium in case of Type–III was somewhat thin and cottony which later became compact to form patchy felty areas while the other portions remained as before. Gradually, shades of colour appeared in all cases. In Type–I, the mycelium at first turned Vinaccous which later deepened to Drab colour in about a month. In Type–II, the mycelium became Pale Pinkish-Cinnamon and remained in that condition during subsequent stages of development. In Type–III, the mycelium turned Pinkish-Buff at places and no further change of colour was observed in this case even in cultures 1-month-old.

B. *Oxidase Tests* (Plate II, figs. 13–18)

Oxidase tests, as originally recommended by Bavendamm (1928) to distinguish a "white rot' fungus from the 'brown rot' type, were also performed. These tests were made in the laboratory by growing polysporous mycelia of the three types of *Polystictus xanthopus* on 2.5% malt-agar containing 0.5% gallic or tannic acid in Petri-dishes. Dark brown rings due to the presence of oxidase appeared in all cases within 36–48 hours indicating that all the three types of mycelia are capable of decomposing lignin constituents of the wood and can be considered positive reactors. Therefore, they are all of "white rot' type. In general, the intensity of reaction was greater on gallic acid medium than that on the medium containing tannic acid due to both Types I and II while the reverse condition prevailed in case of Type–III. The variation in the intensity of reaction and diameters of the diffusing zones after 48 hours have been shown in a comparative way in Table 4. The terms used in describing the intensity of reaction are those of Nobles (1948).

Table 4. *Comparative account of the oxidase activities of the polysporous mycelia of the three types of* Polystictus xanthopus.

		Type–I	Type–II	Type–III
Tannic acid	Diameter	20 mm.	17 mm.	22 mm.
	Intensity	Strong	Moderately Strong	Very Strong
Gallic acid	Diameter	17 mm.	20 mm.	18 mm.
	Intensity	Strong	Strong	Strong

C. *Effects of acidity and alkalinity* (Plate IV, figs. 31–42)

In order to find out whether there was any variation in the rate of growth of the polysporous mycelia of the three types of *Polystictus xanthopus* in a medium having different *p*H, an experiment was set up. The medium taken was 2.5% *malt-agar* and N./10 HCl and N./50 NaOH solutions were used as acid and alkali to prepare four different *p*H grades, viz., 4, 6, 8, and 10, two on the acid side and two on the alkali. A small disc of inoculum was put at the centre of each plate with the mycelial side downwards. The inoculated plates were then kept in diffused light and at room-temperature (28°-32°C.) of the laboratory.

In all cases, except in Type–III, growth started after 24 hours while in Type–III it was evident in about 36 hours. On the fifth day of inoculation the variation in the rate of growth of the three types of mycelia were noted as shown in Table 5.

Table 5. *Comparative data showing the rate of growth in diameter of the three types of mycelia of* Polystictus xanthopus *on the fifth day after inoculation on malt-agar having different p*H.

*p*H (range)	Type–I	Type–II	Type–III
4	5.5 cm.	6.6 cm.	5 cm.
6	6 cm.	5.5 cm.	5.7 cm.
8	4.2 cm.	3.5 cm.	3.5 cm.
10	2 cm.	1.5 cm.	1.7 cm.

From the table it is evident that there is no marked variation in the rate of growth of the three types of mycelia on a medium having different *p*H. There is, however, a decline in the rate of growth in Types I and III on both sides of *p*H 6. In Type–II, on the other hand, this decline in the rate of growth becomes evident even from *p*H 6. That is to say, the growth in diameter of the colonies is maximum in *p*H 6 in Types I and III, while in Type–II it is maximum in *p*H 4. In *p*H 10, the growth of mycelia of all the types is considerably checked.

D. *Hyphal Characteristics*

In all cases preparations for microscopic examination were made of mycelium mounted directly in water and examined with the aid of a Phase-contrast microscope. For making permanent preparations, hyphae from different regions of the mat were mounted in Erythrosine-glycerine-agar jelly. In order to note size, form and gradual differentiation of the actively growing hyphae, observations were made from different regions of 7, 10 and 14-days-old cultures beginning from the periphery to the centre of the mat before it attained tough consistency.

In describing the different types of hyphae, the descriptive terms as proposed by Corner (1932) have been used throughout. In order to avoid unnecessary repetition a full description of the hyphal types and their

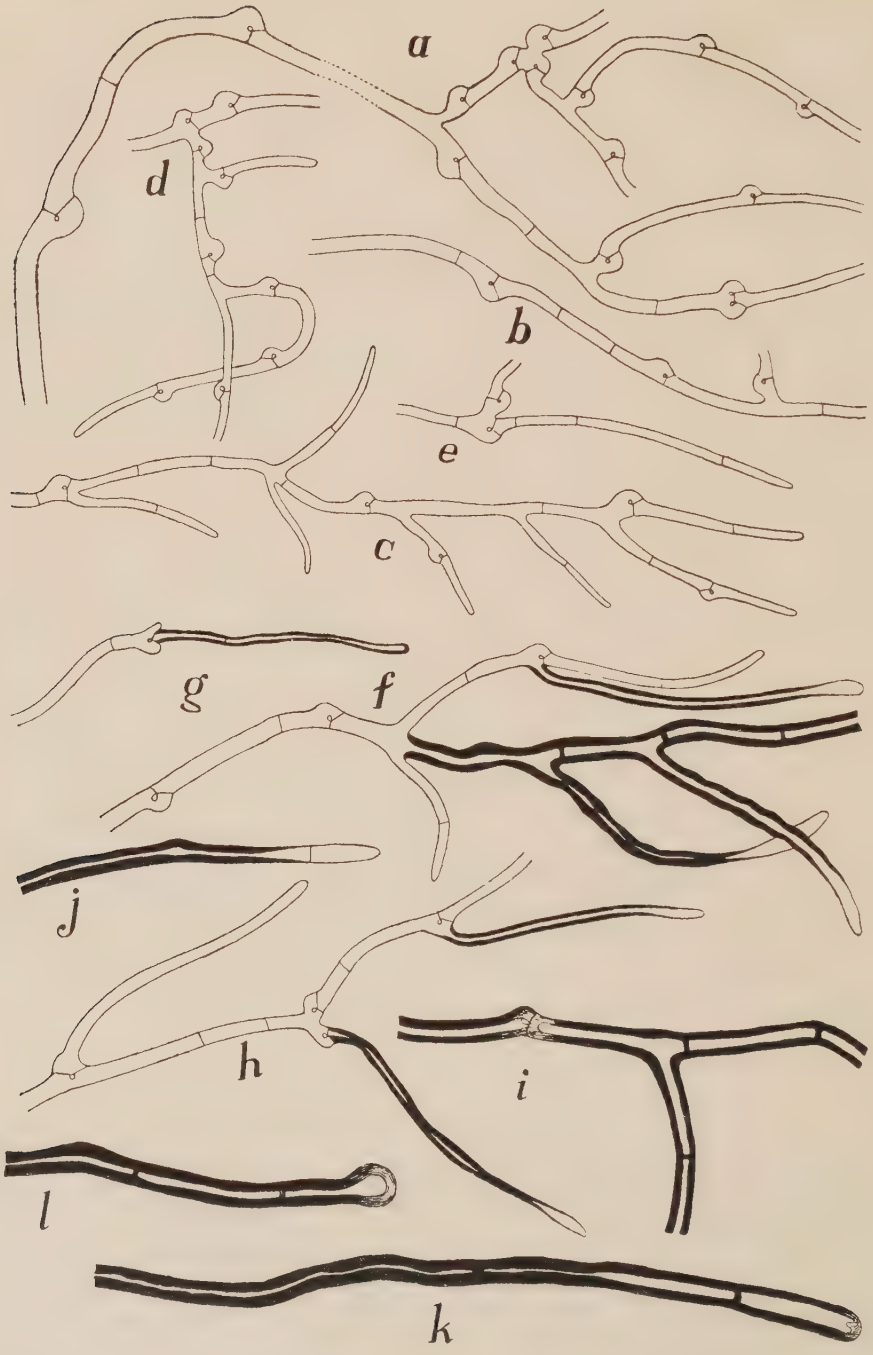
interrelations in the polysporous mycelium of Type–I and the salient descriptive features of the hyphae in the polysporous mycelia of the other two types are given separately.

Type–I (Text-fig. 2)

The hyphae of the advancing zone are of the *generative type* being always thin-walled, hyaline, straight or slightly flexuous, closely or distantly septate, full of granular contents and usually bearing a single clamp-connexion at each septum. Many plain septa are also present. The clamp-connexions are usually found alternately on opposite sides of the same hypha (Text-fig. 2, a–e). Opposite clamp-connexions are rarely found (Text-fig. 2, a). The hyphae are sparingly branched and each branch either originates from the opposite side of a hypha just below a clamp-connexion or the clamp-connexion proliferates and forms the branch. The width of the hyphae is very variable, being 1.5–4 (5) μ. The wider hyphae have been found to contain less granular contents and as such they appear to be more hyaline.

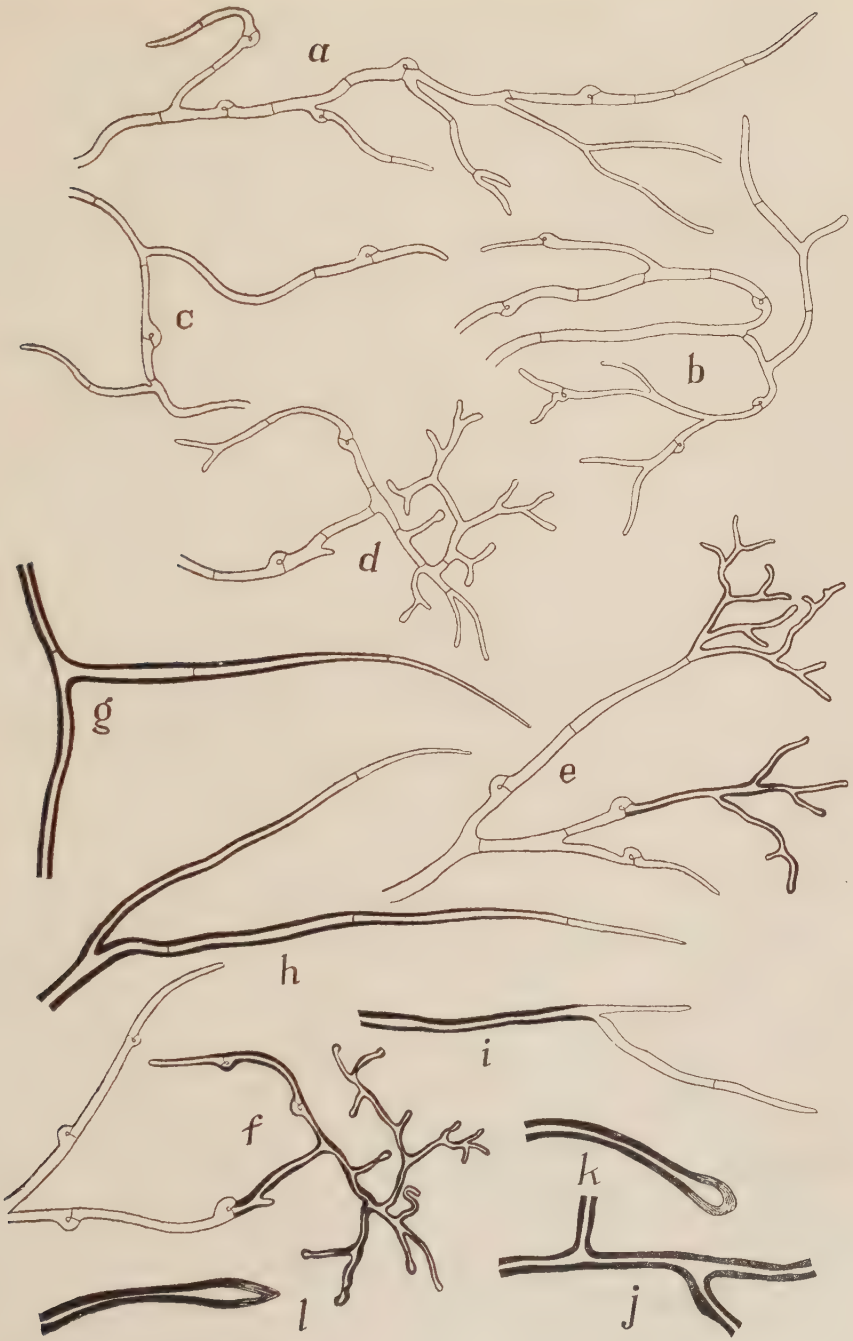
Just in the region a little behind the zone of advance, origin and differentiation of other types of hyphae from the generative type can be well-recognized. The latter type becomes less conspicuous, often branches profusely forming a complicated network and with infrequent clamp-connections. Thick-walled hyphae, mostly of the *skeletal type*, are very abundant at this stage. They are mostly hyaline, always unbranched, straight or slightly flexuous, always without clamp-connexions, aseptate to distantly septate and originating either from the main thin-walled generative hypha on the opposite and just below a clamp-connexion or by its direct proliferation. The thickness of the wall of such a hypha is very variable. It may be uniform or unequeal in the same hypha. The lumen sometimes becomes obliterated at places so that its continuity is broken and the hypha appears to be aseptate. The granular contents become fewer and fewer and sometimes appear to be almost absent. The width of the hypha varies between 2–6 μ. The tip of the skeletal hypha is attenuated or rounded, may be thick-walled or thin-walled and sometimes slightly inflated (Text-fig. 2, g-h, j-l).

Some thick-walled hyphae appear to be distantly or sparingly branched, and consitute the *mediate type* (2.5–3.5 μ). They are undoubtedly derived from the generative hyphae like the skeletal type but they are branched and without clamp-connexions. Sometimes they are formed due to direct transformation or parts of generative hyphae which do not bear clamp-connexions from the beginning. They may also be formed by the thickening of the walls of the branched generative hyphae whose clamp-connexions become totally obliterated due to thickening and stretching or by their proliferation. All gradations between generatve and mediate types of hyphae are noticeable (Text-fig. 2, f, i). In the older portions and towards the centre of the mat, all the three types of hyphae, namely, generative, skeletal and mediate, can be recognized but of these, the generative type predominates. Some of the generative hyphae are distinctly



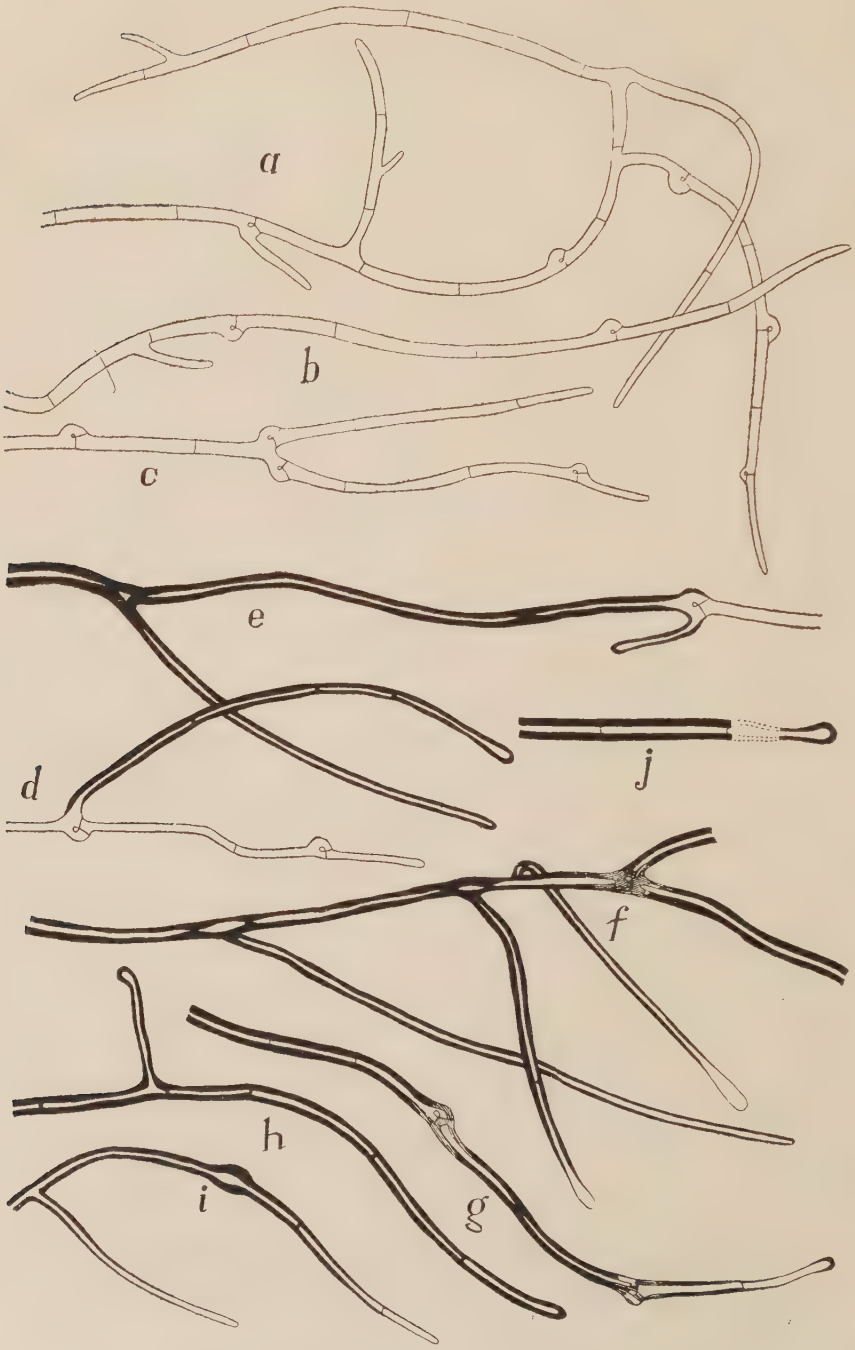
Text-fig. 2. Hyphal system in the polysporous mycelium of *Polystictus xanthopus* (Type–I). × 1400.

(For explanation, vide text)



Text-fig. 3. Hyphal system in the polysporous mycelium of *Polystictus xanthopus* (Type–II). × 1400.

(For explanation, vide text)



Text-fig. 4. Hyphal system in the polysporous mycelium of *Polystictus xanthopus* (Type–III). × 1400.

(For explanation, vide text)

thick-walled and show plenty of proliferating clamp-connexions. The *binding hyphae*, so characteristic of the fructifications of this type, are not in evidence even in old cultures.

Type–II (Text-fig. 3)

As in Type–I, the advancing zone is full of *generative hyphae* (1–3.5 μ) with abundant simple clamp-connexions and also with plain septa (Text fig. 3 a–d). Partial or entire transformation of some of the generative hyphae into thick-walled branched hyphae with or without clamp-connexions can be clearly seen. This may be designated as much branched *mediate hyphae* and their width varies between 1–2 (4) μ (Text-fig. 3, e, f). Some of the generative hyphae appear to be entirely empty, closely septate and without clamp-connexions. No skeletal hyphae have been differentiated in this region.

In the regions behind the advancing zone and towards the centre, three types of hyphae, viz., generative, mediate and skeletal, become gradually differentiated. The *skeletal hyphae* [1.7–2.8 (4) μ] in the older region of the mat become most abundant. The *generative hyphae* are fewer and there are transitional stages between these and the *mediate type* (Text-fig. 3, e, f). The hyphal characteristics are more or less similar to those in Type–I, but the apices of the skeletal and those of the branches of mediate hyphae (1–2.5 μ) upto a considerable distance from the tip, remain thin-walled and become gradually attenuated showing nuclei and sparse granular contents within (Text-fig. 3, g, i). The skeletal and mediate hyphae (Text-fig. 3, i-l) are usually uniformly thickened showing more or less linear lumina. Partially twisted or coiled binding hyphae which are present in the fructification of this type cannot be observed.

Type–III (Text-fig. 4)

The same three types of hyphae, viz., *generative*, *skeletal* and *mediate*, enter into the composition of the mycelium of this type. The advancing zone is similar in having only the characteristic generative hyphae (Text-fig. 4, a-c). The width of the generative hyphae vary between 1.5–3.5 μ. The clamp-connexions are comparatively few and the plain septa are frequent. Just behind the zone of advance, only the skeletal hyphae become differentiated from the generative type and they become more and more abundant towards the centre (Text-fig. 4, d, g, i, j). Their characteristics are similar to those found in Type–I. In the central portion of the mat, all the three types of hyphae, viz., generative, skeletal and mediate, are present of which the skeletal type (2–5 μ) predominates. The mediate hyphae (2–5 μ) are springly branched (Text-fig. 4, e-f, h) and agree closely with those in the other two types.

Antagonism and Compatibility

The nature of antagonism between the polysporous mycelia of the three types of *Polystictus xanthopus* have been ascertained. They were tested on 2.5% *malt-agar* slants in special culture-tubes ($10'' \times 1\frac{1}{2}''$). The

three types of mycelia were paired in all possible combinations. All pairings were run in triplicate and each set repeated at least once. For observing the uniting hyphae *in situ* pairings were made on cleared *malt-agar* (1%) in Petri-dishes. When the colonies approached each other, the lids of the Petri-dishes were removed and they were directly observed under the microscope.

The mycelia of the three Types show diversity in their reaction in culture which ranges from complete intermingling to apparent antagonism at the point of meeting. The three principal types of reaction observed are: (a) the two colonies either intermingle completely without any apparent antagonism and any line of demarcation between them, (b) the two colonies do not intermingle freely and are apprently separated from each other by a narrow space between them, and (c) two such colonies although intermingle freely yet show a faint line of separation in between them. Close examination of these zones of separation reveals that these are not entirely free from the hyphae of the two interacting aerial mycelia and this is always constant for any pair of cultures.

The advancing hyphae of the two colonies when directly observed under the microscope, it has been found that the hyphae of the interacting mycelia simply cross one another, grow in opposite directions and in no case hyphal fusions have been observed in the early stages. Further observations could not be made during subsequent stages of development due to free intermingling of the hyphae in all directions and the place of contact between the two colonies became somewhat dense.

Decay-Resistance

In order to determine the rate of decay of sapwood samples of Sāl (*Shorea robusta*) caused by the mycelia of three different forms of *Polystictus xanthopus* decay tests were done in the laboratory. The process in general involved in exposing small sterilized smaples of sound sapwood of Sāl to the attack of the mycelia of three different forms of the fungus growing in pure cultures under a set of controlled conditions and the resulting weights of decay were measured by estimating the loss in dry weight. The results also give an indication of the degree of decay-resistance of sapwood of Sāl to the attack of each of the three types of mycelia under consideration. Although various methods on durability tests of different timber were suggested by various workers the writers have followed mainly the method proposed by Banerjee (1956) with some modifications as detailed below.

Small rectangular wood blocks ($1'' \times \frac{1}{2}'' \times \frac{1}{2}''$) from sound sapwood of *Shorea robusta* were cut uniformly with their long axis parallel to the grain of the wood. These were plained, numbered serially and then dried in an oven at 60°C. to a constant weight. Before sterilization the test-blocks were soaked in distilled water till the moisture-content of each test-piece was 65–70%. This was done in order to ensure proper water supply to the mycelium of the fungus for its activity during the test-period

and it was much above the fibre-saturation-point, (Cartwright and Findlay 1946). Sets of four such test-pieces were introduced into several test-tubes ($10'' \times 1\frac{1}{2}''$) each having a little distilled water at the bottom, plugged and sterilized for ten minutes at 15 lbs. pressure (Banerjee, 1956).

About twelve days before exposing the sterilized test-samples to the mycelia, small bits of actively growing polysporous mycelium of each type were transferred separately to sterile 2% *malt-agar* slants in Kolle flasks. Each flask contained about 80 c.cm. of the medium which was slanted after sterilization. Within a week the mycelium of each flsak almost covered the surface of the slant and in about twelve days was ready to receive the test-pieces. At this stage the sterilized wood-blocks were introduced into the Kolle fiasks, four in each, and placed over the actively growing mycelium of the fungus. Twelve such test-pieces were exposed to the mycelium of each type. Adequate controls were also prepared in a similar way but without the fungus. These inoculated flasks with controls were kept in darkness and room temperature (28°–32°C.) of the laboratory during the experimental period.

In order to obtain a significant loss in weight, the period of exposure to fungal attack, in this case, was four months following Cartwright and Findlay's (1946) recommendations. According to them this period is sufficient in order to obtain a fair indication of the rate of decay of wood-samples. At the end of the test-period, the wood-blocks were taken out of the flasks and the superflcial mycelia were carefully removed without causing injury to the blocks. Immediately, the initial weights of these blocks were first noted and these were then again dried to a constant weight as before at 60°C. The resulting dry weights were subtracted from the original and the percentage losses based on dry weights of the sound blocks were calculated. Average moisture-content of the test-pieces before and after they were exposed to fungal action were also determined in the same way. The results of the experiment are given in Tables 6-8.

Table 6. *Decay-resistance of sapwood of* Shorea robusta *after 4-months-test when exposured to the polysporous mycelium of* Polystictus xanthopus (Type–I)

Serial number of blocks	Initial oven-dry weight (gm.) before test	Final oven-dry weight (gm.) after test	Loss	Loss %
1	3.160	2.415	0.745	23.2
2	3.730	3.390	0.340	9.1
3	3.505	3.025	0.480	13.6
4	3.210	2.285	0.925	28.8
5	3.177	2.750	0.427	13.4
6	3.777	2.852	0.925	24.3
7	3.237	2.170	1.067	32.3
8	3.750	2.985	0.765	20.4
9	3.470	2.915	0.555	15.9
10	3.485	3.175	0.310	8.9
11	3.142	2.440	0.702	22.3
12	3.527	2.910	0.617	17.5

Average loss in dry weight (%) = 19.1

Table 7. *Decay-resistance of sapwood of* Shorea robusta *after 4-months-test when exposed to the polysporous mycelium of* Polystictus xanthospus (Type–II)

Serial number of blocks	Initial oven-dry weight (gm.) before test	Final oven-dry weight (gm.) after test	Loss	Loss %
1	3.077	2.670	0.407	13.2
2	3.435	3.220	0.115	3.3
3	3.155	2.760	0.395	12.5
4	3.532	3.200	0.332	9.4
5	3.755	3.625	0.130	3.5
6	3.462	3.305	0.157	4.4
7	3.665	3.415	0.250	7.3
8	3.930	3.285	0.645	16.4
9	3.857	3.740	0.117	3.1
10	3.115	2.640	0.475	15.2
11	3.655	3.205	0.450	12.6
12	3.300	2.920	0.380	11.5

Average loss in dry weight (%) = 9.36

Table 8. *Dacay-resistance of sapwood of* Shorea robusta *after 4-months-test when exposed to the polysporous mycelium of* Polystictus xanthopus (Type–III)

Serial number of blocks	Initial oven-dry weight (gm.) before test	Final oven-dry weight (gm.) after test	Loss	Loss %
1	3.467	3.025	0.442	2.7
2	3.350	3.285	0.065	1.9
3	3.980	3.645	0.245	5.2
4	3.535	3.180	0.355	10.0
5	3.637	3.492	0.145	3.9
6	3.552	3.420	0.132	3.8
7	3.542	3.320	0.222	6.2
8	3.357	3.175	0.182	5.4
9	3.285	3.150	0.135	4.1
10	3.690	3.460	0.230	6.2
11	3.722	3.605	0.117	3.1
12	3.885	3.550	0.335	8.5

Average loss in dry weight (%) = 5.0

From the Tables, it is clear that the average loss in dry weight of sapwood blocks is maximum in Type–I where it is 19.1% and those in Type–II and Type–III are 9.3% and 5.0% respectively, after 4-months-test. Thus, the losses in the sapwood blocks due to mycelial infection of Types II and III are very little in comparison to that in the sapwood blocks caused by the polysporous mycelium of Type–I. Findlay (1938) has classified the timbers into five groups according to their natural resistance to fungal decay on the basis of a four-months-test. Here, the decay of sap-wood caused by Type–I comes under Findlay's 'non-resistant' group as the average loss in dry weight in the sapwood due to this type is within 10–30%. On the contary, the sapwood can be termed 'moderately resistant' and

'resistant' to the attacks by the Types II and III as the former causes average loss in weight within 5–10% and the latter within 5% during 4-months-test. Thus, it is evident that the sapwood of *Shorea robusta* greatly differ in their degrees of natural resistance to deacy caused by the polysporous mycelia of the three types of *Polystictus xanthopus*. In this connection, it has been noted that in all cases the controls show very little or no loss of weight after the experimental period.

After the test-period, the average moisture-content of the sapwood blocks was again determined. It was found that it came down from 65–75% to 40%. No attempt was, however, made to control the exact moisture-content of the wood-blocks during the test-period. Nevertheless, it clearly indicates that the moisture-content in general remained above the fibre-staturation-point and consequently it was sufficient for the normal activity of the mycelia of the three types of *Polystictus xanthopus* in bringing about the losses.

Reactions to Zinc chloride

In order to determine whether the polysporous mycelia of the three types of *Polystictus xanthopus* react differently to a toxic substance or not, toxicity tests were made by using zinc chloride as the toxic substance. This inorganic salt, a common wood preservative, was selected because of the ease of handling and it could be easily used with an agar medium in Petri-dishes. It is well known that the toxicity of a wood-preservative is one of the important factors influencing its effectiveness on wood and this property can be readily evaluated by making laboratory tests. There are two outstanding methods for carrying out toxicity tests. The American workers prefer the use of an agar medium in which to test the wood-preservative while the European and other methods favour the use of wood as the medium with which the wood-preservative is tested. Since the present investigation is not for evaluating the effectiveness of various preservatives and their toxicity to fungi, the writers have preferred to follow the former method by using a malt agar medium to which zinc chloride as an antiseptic was added.

For this purpose by adding requisite quantities of sterile zinc chloride solution in distilled water (1%) to sterilised *malt-agar* medium (2.5%) in Petri-dishes the folliwng concentrations (%) of zinc chloride were obtained: 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1. The Petri-dishes were inoculated with the mycelia of all the three types and were allowed to grow for a week in ordinary conditions of diffused light and temperature (28–30°C.) of the laboratory. After the test-period, it was found that only the growth of the mycelium of Type–III was completely checked (Plate III, figs. 27–30) in the medium containing 0.1% zinc chloride while others remained almost unaffected excepting in that the rate of growth in Type–I became slightly slower.

Consequently, subsequent trials were given with a higher concentration of zinc chloride using 2% sterile stock solution and the grades were

0.1, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19 and 0.2 per cent. Similarly, the plates were inoculated with mycelia of Types I and II. After 7-days-test, the growth of the mycelium of Type–I was only inhibited completely in 0.16% (Plate III, figs. 19–22) concentration of zinc chloride while the mycelium of Type–II although not inhibited completely in these concentrations yet showed a gradual decline in the rate of growth from concentration 0.16 to 0.2 per cent (Plate III, figs. 23–24).

Finally, in order to determine the point of inhibition of the mycelium of Type–III similar tests vere performed with still higher concentrations of zinc chloride (0.22, 0.24, 0.26 and 0.28 per cent) using 3% sterile stock solution. After a week's test, it was found that the growth of the mycelium was completely checked in 0.24% concentration of zinc chloride (Plate III, figs. 25–26).

Thus, the reactions and the power of tolerance of the mycelia of the three types to a toxic substance are different for each type which appear to be maxiumum in Type–II, minimum in Type–III while the mycelium of Type–I may be placed in the intermediate category.

Discussion

A comparative study of the different forms of *Polystictus xanthopus* recognized by the writers as Types I, II and III, makes it possible to discuss in a general way some of the salient points regarding the relationship that exists between them. From the previous literature, it appears that there exists two varieties of *Polystictus xanthospus* (here recognized as Types II and III) which not only differ from each other but also from the typical form (Type-I) in the character of the hymenial surface. Closer examination of the three types of fructification of the fungus and their subsequent study, however, reveal that this separation is somewhat justified. This separation into three types by macroscopic characters alone cannot, however, be substantiated in all cases by microscopic characters and in other details. From the thickness of the context, the length of the pore-tubes and the width of the dissepiments, it can be stated that all the three types vary greatly from one another but in the diameter of the pore-tubes, Type–I resembeles closely Type–II while Type–III forms a separate group possessing much wider pores. The dimensions of the basidia and basidiospores in Types I and II are also more or less the same but they are much bigger in Type–III.

The hyphal composition of the fructiacations of Types I and II appear to be similar in having all the four types of hyphae, viz., *generative*, *skeletal*, *mediate* and *binding*. In Type–II, on the other land, the binding hyphae are totally absent. There are, however, some much branched generative hyphae which look somewhat like the binding hyphae and these seem to be the transitional stages between the generative and the binding types. Further, the widths of the different types of hyphae of the fruit-bodies of Types I and III vary within limits while those of Type–II exhibit a wider range.

As regards the growth characteristics on agar medium, there exists slight variation in texture, colour and rate of growth between the mycelia of all the three types. The mycelia of Types I and II are fast growers at the onset while that in Type-III, the initiation of growth is much delayed. During the later stages, however, the daily increment of growth in all the three types, is more or less the same. This is also seen in wood-block cultures of all the three types in which the growth characteristics in Types I and II have been found to resemble more or less closely with each other while that in Type–III is less pronounced and the mycelium exhibits poor growth. There is also a difference in reaction to oixdase tests, although all the types are positive reactors. The Types I and II, differing only in their intensity of reaction, behave more or less similarly on both gallic and tannic acid media. In each case, however, the reaction appears to be more pronounced in gallic acid medium than that in the other. In Type–III, on the other hand, the reaction is much more vigorus in tannic acid medium.

From a comparative study of the effects of the variation of *p*H on growth of the three types of mycelia, it can be said that the difference in *p*H of the medium has but very little effect on the rate of growth of the colonies. As alkalinity of the medium increases to *p*H 10, growth in all cases in considerably checked. On the acid side (*p*H 4) the growth of the colonies is also slowed down excepting that in Type—II The maximum growth in the diameter of the colony has been observed in *p*H 6 in both Types I and III whereas, in Type–II, there is a gradual decline in growth of the colony from *p*H 6–10.

The mycelia of all the three types are composed of *generative, skeletal* and *mediate* hyphae only. The *binding* hyphae which are characteristic of the fructifications of Types I and II, are not differentiated at this stage. Further, the modes of development of the skeletal and mediate types from the generative hyphae are identical in all cases. In other minor details, slight differences between them do occur but these appear to be of not much significance.

From the decay-resistance tests it has been found that the three types differ markedly in their ability to decay the sapwood of Sāl. Of these, Type–I appears to be most active in its ability to decay sapwood and as such the wood falls under the 'non-resistant' group. In other cases the sapwood of Sāl is 'moderately resistant' and 'resistant' to the attacks by Types II and III respectively.

In the power of tolerance of toxic substance like zinc chloride, the three types also differ from one another. The growth of the mycelium in Type–III is totally checked in 0.1% of zinc chloride and has been found to be the weakest of all the three types in so far as their power of tolerance is concerned. On the other hand, the power of tolerance is maximum in Type–II where the inhibition point is 0.22%. Type–I falls under the intermediate category, the point of inhibition being 0.14%.

Pairing experiments with the polysporous mycelia of all the three types reveal that their interaction varies from complete intermingling to

apparent inhibition of growth at the place of contact between the two reacting mycelia. The Types I and II have been found to be apparently antagonistic to each other while it is homogeneous without any antagonism between mycelia of Types I and III. The reaction between the mycelia of Types II and III is intermediate with slight tendency towards antagonism and showing a faint line of aversion between them. In all cases, however, free intermingling of hyphae takes place but in no case hyphal fusion between the uniting pairs can be observed. Such intersterility between strains, varieties or forms of the same species is, in no way, uncommon as reported by Verrall (1934) in *Fomes igniarius*, Arnold (1935) in *Collybia cirrhata* and Biggs (1937) in *Corticium coronilla*. These are regarded as collective species in which distinct intersterile forms occur and these are further characterised morphologically. This condition also possibly holds good for *Polystictus xanthopus*.

It is often suggested that these differences are due to altitudinal variations which seems to be so untenable, as fructifications of all the three types have been collected from the same locality and growing under identical conditions. Thus, variations within this particular species may possibly be attributed to genetical or other factors rather than ecological ones.

Reference

Arnold, J. D. (1935). A comparative study of *Marasmius* and *Collybia* in culture. *Mycologia*, **27**, 388–417.

Bagchee, K. and Bakshi, B. K. (1950). Some fungi as wound-parasites on Indian trees. *Indian For.*, **76**, 244–253.

Bakshi, B. K. and Bagchee, K. (1950). Principal diseases and decays of Oaks in India. *Indian Phytopathology*, **3**, 124–139.

Banerjee, S. N. and Bakshi, B. K. (1945). Studies in the biology of wood-rotting fungi of Bengal. *J. Ind. Bot. Soc.*, **24**, 73–93.

Banerjee, S. N. (1947). Funguous flora of Calcutta and Suburbs. *Bull. Bot. Soc. Beng.*, **1**, 37–54.

Banerjee, S. N. (1956). A disease of Norway spruce (*Picea excelsa* (Lam.) Link) associated with *Stereum sanguinolentum* (A, et S.) Fr. and *Pleurotus mitis* (Pers.) Berk. *Ind. J. mycol. Res.*, **1**, 1–30.

Banerjee, S. N. and Samadder, K. R. (1957). Studies on Heterothallism—II. *Polystictus xanthopus* Fr. *Sci. & Cult.*, **22**, 578–579.

Bavendamm, W. (1928). Neue Untersuchungen Über die Lebensbedingungen holzzerstörender Pilze. *Zbl. Bakt.*, 11, **76**, 172–277.

Biggs, R. (1937). The species concept of *Corticium coronilla*. *Mycologia*, **29**, 686–706.

Bisby, G. R. (1945). *An Introduction to the Taxonomy and Nomenclature of Fungi*. *Imp. Mycol. Inst.*, Kew, Surrey, England.

Bose, S. R. (1919). Polyporaceae of Bengal VI. *Rep. Ind. Assoc. and Proc. Sci. Con.*, VI.

Bose, S. R. (1929). Revival of an old fruit-body of *Hexagonia discopoda* Pat. and Har. and successful spore-culture from its fresh spore discharge. *Ann. Mycol.*, Berl., **27**, 321–323.

Bose, S. R. (1929*a*). Artificial culture of *Ganoderma lucidus* Leyss from spore to spore. *Bot. Gaz.*, **87**, 665–667.

Bose, S. R. (1930). Biology of Wood-rotting fungi common in forest areas. *J. Linn. Soc. (Bot.)*, **48**, 417–438.

Bose, S. R. (1936). A note on the variation of pores of *Polystictus xanthopus* Fr. and *Polystictus flabelliformis* Kl. at high altitudes. *Trans. Brit. mycol. Soc.*, **20** (2), 185.

Butler, E. J. and Bisby, G. R. (1931). *The Fungi of India. Sci. Mono.* No. 1, *I.C.A.R.* New Delhi, India.

Cartwright, K.St. G. and Findlay, W. P. K. (1946). *Decay of Timber and its Prevention. H.M.S.O*, London,

Corner, E. J. H. (1932). The fruit-body of *Polystictus xanthopus* Fr. *Ann. Bot. Lond.*, **46**, 71–111.

Findlay, W. P. K. (1938). The natural Resistance to Decay of some Empire Timbers. *Emp. For. J.*, **17**, 249–259.

Fritz, C. W. (1923). Cultural criteria for the distinction of wood-destroying fungi. *Trans. Roy. Soc. Can.*, V, **17**, 191–288.

Killermann, S. (1928). *Die naturlichen pflanzenfamilien*, **6**, 186.

Lloyd, C. G. (1910). *Synopsis of the sections Microsporus, Tabacinus and Funales of the genus Polystictus*, **3**, 49–94, *Cincinnati, Ohih.*

Lloyd, C. G (1921). *Mycological Notes*, No. 65, 1043, Cincinnati, Ohio.

Nobles, M. K. (1948). Studies in Forest Pathology VI. Identification of cultures of wood-rotting Fungi. *Can. J. Res.*, C, **26**, 281–431.

Overholts, L. O. (1929). Research methods in the Taxonomy of Hymenomycetes. *Proc. Int. Cong. Plant. Sci.*, **2**, 1688–1712.

Petch, T. (1916). A preliminary list of Ceylon Polypori. *Ann. Roy. Bot. Gardn.* Peradeniya, **6** (2), 128.

Ridgway, R. (1912). *Colour standard and Colour Nomenclature*, Washington, D.C.

Saccardo, P. A. (1888). *Sylloge fungorum omnium hucusque cognitorum.*, **6**, 215.

Schmitz, H. (1925). Studies in wood-decay V. Physiological specialization in *Fomes pinicola* Fr. *Amer. J. Bot.*, **12**, 163–177.

Stakman, E. C. (1929). Physiological specialization in Pathogenic fungi. *Proc. Int. Cong. Plant. Sc.*, **2**, 1312–1330.

Verrall, A. F. (1934). Variation in *Fomes igniarius* (L.) Gill. *Minnesota Tech. Bull.*, **117**, 1–41.

(Received for publication 17 February 1957)

EXPLANATION OF PLATES I–IV.

Plate I

Figs. 1–6. The fructifications of *Polystictus xanthopus* showing upper and hymenial surfaces : Type–I (figs. 1 and 2), Type–II (figs. 3 and 4) and Type–III (figs. 5 and 6). Half Nat. size.

Plate II

Figs. 7–9. Habit of growth of polysporous mycelia of *P. xanthopus* 10-days-old, on *malt-agar* at 28°–30°C. in darkness : Type–I (fig. 7), Type –II (fig. 8) and Type–III (fig. 9).

Figs. 10–12. Cultures of polysporous mycelia of three types of *P. xanthopus*, about 1-month-old, on sapwood-blocks of *Shorea robusta* at 25°–30°C. in darnkness : Type–I (fig. 10), Type–II (fig. 11) and Type–III (fig. 12).

Figs. 13–18. Oxadase tests with the polysporous mycelia of Type–I (figs. 13, 16), Type–II figs. 14, 17) and Type–III (figs. 15, 18) of *P. xanthopus* on *malt-agar* containing 0.5% tannic acid (figs. 13–15) and 0.5% gallic acid (figs. 16–18), after 48 hours of inoculation.

PLATE III

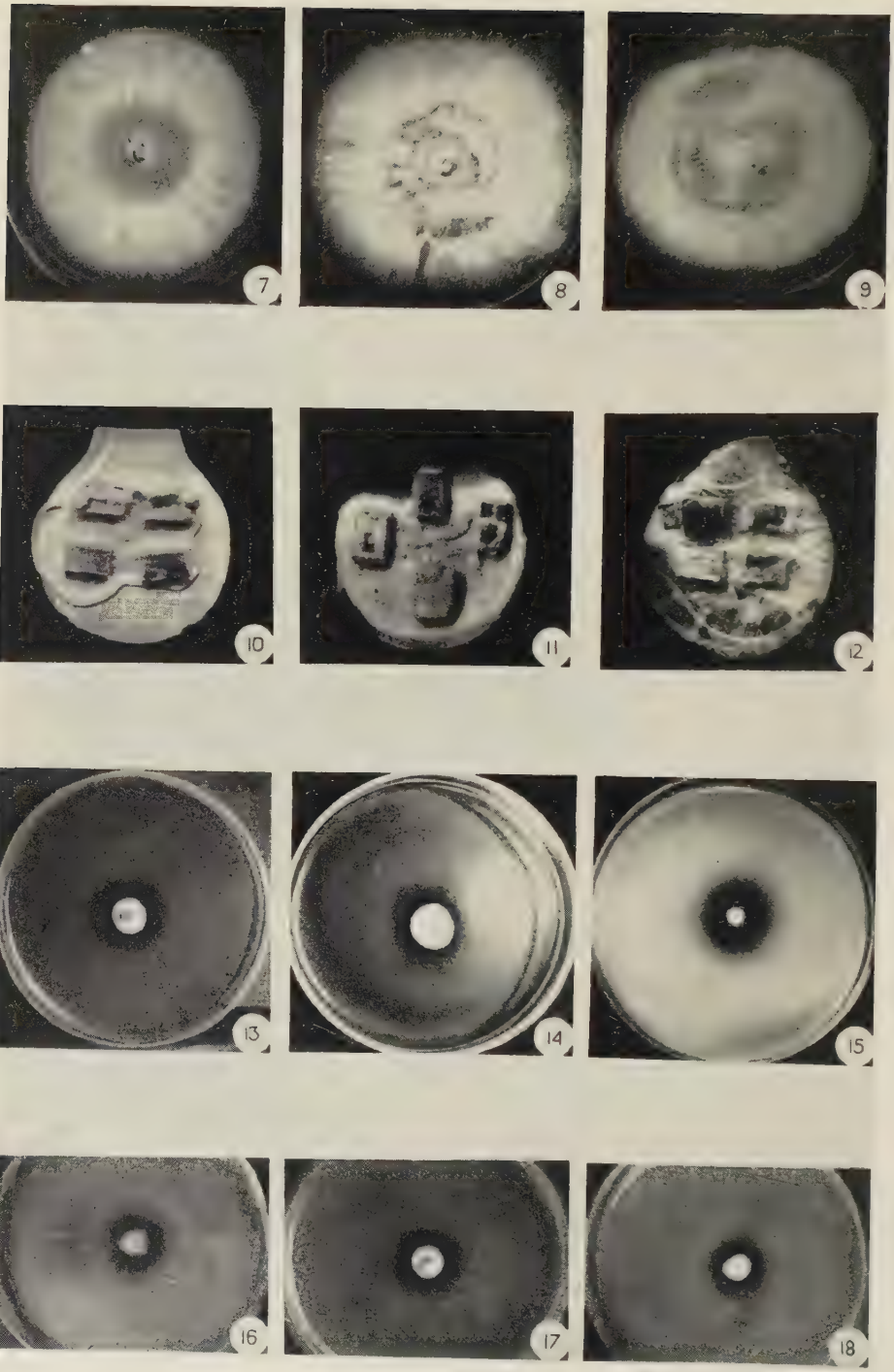
Figs. 19–30. The toxic effect of zinc chloride in *malt-agar* on polysporous mycolia of Type–I (figs. 19–22), Type–II (figs. 23–26) and Type–III (figs. 27–30) of *P. xanthopus* at 28°–30°C. and in the diffused light of the laboratory.

PLATE IV

Figs. 31–42. Effects of acidity and alkalinity of *malt-agar* medium on habit of growth of polysporous mycelia of Type–I (figs. 31–34), Type–II (figs. 35–38) and Type–III (figs. 39–42) of *P. xanthopus* at 28°–32°C. and in the diffused light of the laboratory after five days of inoculation.

Variation in P. xanthopus

S. N. Banerjee & Himani Debi



Variation in P. xanthopus

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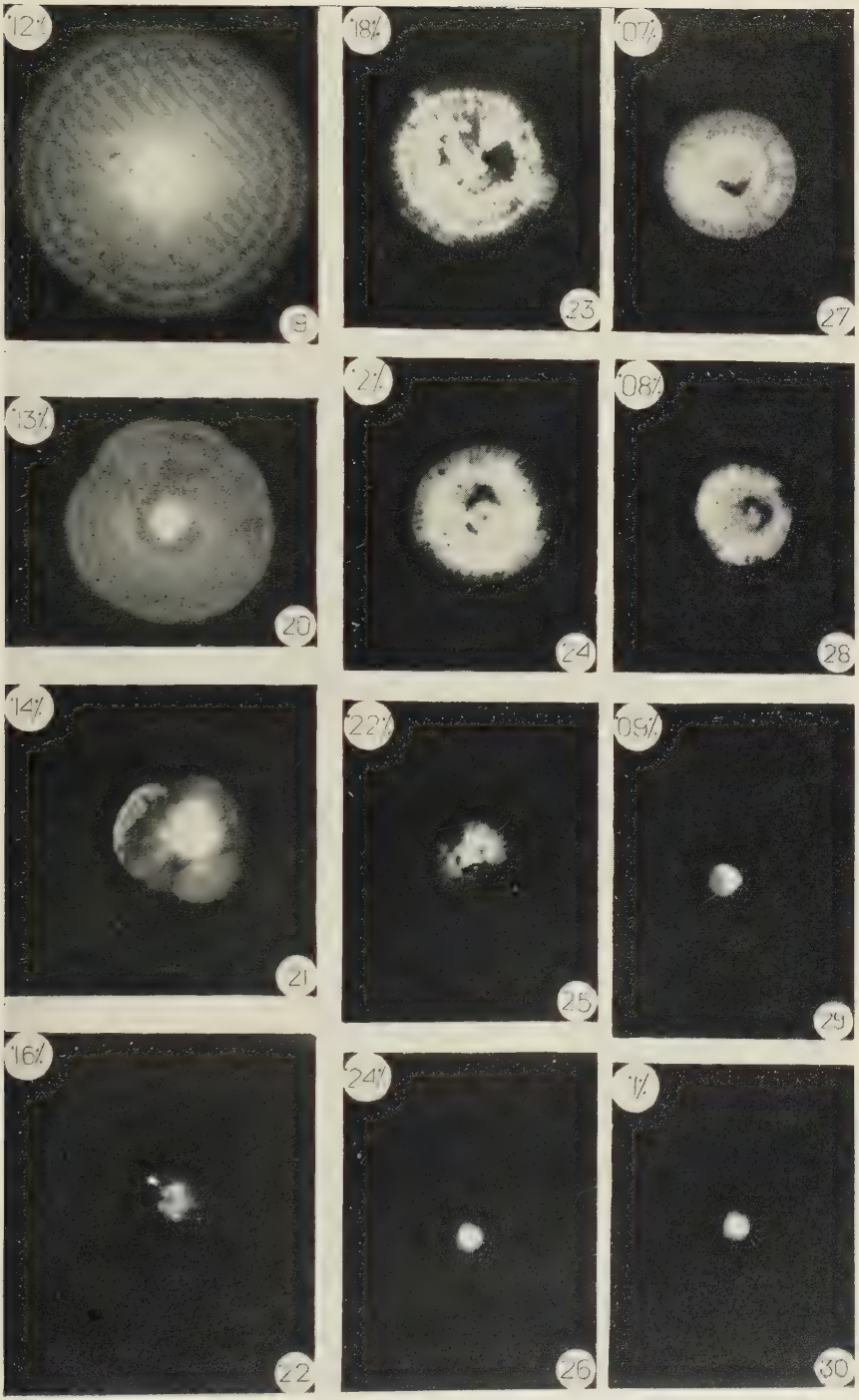

Variation in P. xanthopus

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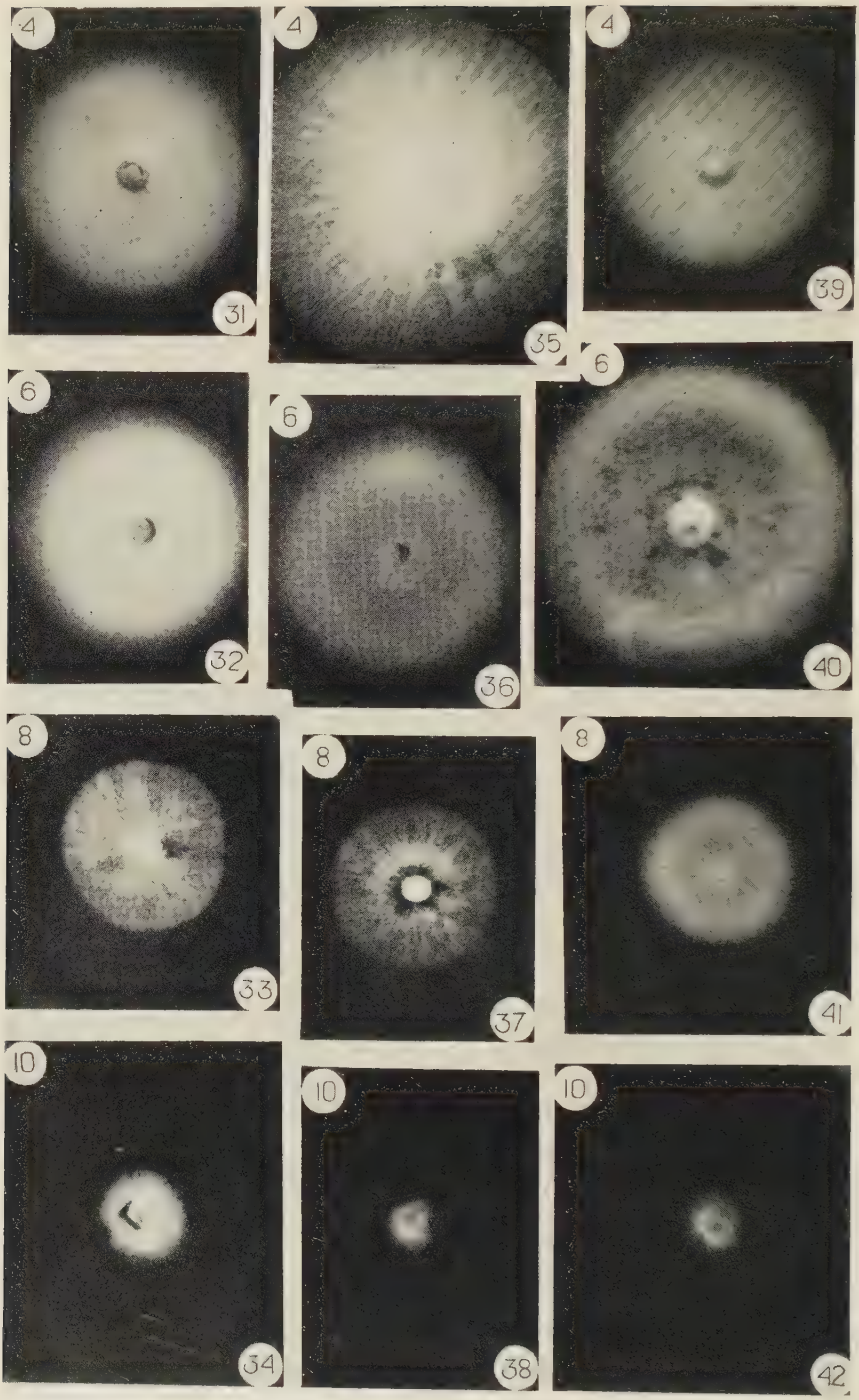

Variation in P. xanthopus

S. N. Banerjee & Himani Debi

Ind. J. mycol. Res., **2** (1 & 2), 29–36 (1956)

A NEW FACTOR IN THE STUDY OF NITROGEN FIXATION IN SOIL

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The nitrogen fixation was found to be greater in the farmyard manured plots than the corresponding unmanured ones in both the fields, one with a heavy alkaline clay soil and the other with a light sandy acid soil. The amount of fixation showed considerable variation with the quantity of the inoculum; with a smaller quantity of the inoculum, the fixations were identical in two plots of the same field while with a larger quantity, fixations increased with the farmyard manured plots and decreased with the unmanured ones. The fixation by a small amount of the inoculum appeared to indicate the nitrogen fixing capacity of the strain while a larger amount reflected the nitrogen fixing index of a soil.

The nitrogen fixation was influenced by other micro-organisms besides the nitrogen fixing flora, whereas the physico-chemical character of soil appeared to have no effect The nitrogen fixation in soil showed variation with the change of season, the activity being low during winter months.

Introduction

The discovery of the phenomena of nitrogen fixation, ammonification, nitrification, carbon dioxide evolution and other microbiocal activities in soil gave an impetus to determine their corelation, if any, with the property of a soil or its productivity. The study of nitrogen fixation in soil as an index of soil fertility received considerable attention in this respect but the results so far had been inconclusive. Brown (1915) found a definite corelation between the nitrogen fixing property of a soil and its yield; Given and others (1916-17) obtained a similar result with one set of experimental plot but not in another; Waksman (1924) likewise could not find any definite co-ordination.

In the present paper, the nitrogen fixation in different types of soil under different manural treatment, have been examined to study the effect of the quantity of inoculum and certain other factors, and the results appear to throw a new light on certain aspect of nitrogen fixation in soil.

Materials and Methods

The soils used were obtained mainly from two different localities, *viz.*, Barnifield at Rothamsted and Stackyard at Woburn. The former is a heavy alkaline clay soil with a *p*H varying from 7.1–7.3, while the latter is a light acid soil with a *p*H range between 5.8–6.0. From each of the fields two plots, a farmyard manured and an unmanured, were selected for the investigation.

The physical character of the different plots and their annual yields are respectively given in Tables 1 and 2.

Table 1 *Physical character of soil (in mg.)*

	Barnfield		Stackyard	
	FYM	X	FYM	X
Coarse sand	13.8	8.3	50.0	58.6
Fine sand	36.4	39.3	24.3	18.8
Silt	21.7	23.7	8.5	12.0
Clay	22.3	27.5	13.3	9.5
Carbonates	0.5	0.3	0	0
Air dry moisture	3.8	3.6	1.3	1.1
Lost by solution	0.9	0.5	0.9	0.1
Difference	0.6	+3.2	1.7	−0.4
Total	100	100	100	100
Loss on ignition	7.8	5.1	3.9	3.7

Farmyard manured = FYM Unmanured = X

Table 2 *Annual yields of mangold root (Tons per acre) and barley grain (cwts. per acre).*

		Barnfield (Mangold root)		Stackyard (Barley grain)	
		FYM	X	FYM	X
Year	1929	18.14	10.85	16.87	9.19
	1930	15.39	3.37	9.50	5.13
	1931	29.10	15.80	8.98	4.02

Farmyard manured = FYM Non-manured = X

Before taking samples, the surface soil of about an inch was first scraped off and six borings to a depth of about 4 inches were taken at random from the different parts of a plot. Samples were then thoroughly mixed and passed through a 3 mm. seive.

Two methods are generally used for estimating the nitrogen fixing capacity of a soil, the soil method and the solution method. The soil method is an approach to a more natural condition, but the results are often unsatisfactory as the amount of nitrogen fixation in this method, is mainly determined by the soil reaction; soil with a *p*H value below 6.0 fixes very little or no nitrogen (Waksman, Karunakar, 1924). The following solution method was, therefore, adopted in these experiments.

50 c.c. amounts of the medium containing Mannitol 10.0 gm., K_2HPO_4 0.5 gm., $MgSO_4$ 0.2 gm., NaCl 0.2 gm., $FeCl_3$ and $MnSO_4$—a trace and distilled water 1 l., and *p*H adjusted to 7.0 were taken in 250 c.cm. Erlenmeyer flasks, sterilized and inoculated with different quantities of soil. About 0.05 gm. calcium carbonate were added to each flask, and incubated for a period of 30 days. The total nitrogen was determined by the Kjeldahl process.

Experimental

(*i*) *Nitrogen fixation in manured and unmanured plots*

A series of experiments were conducted to determine variation in nitrogen fixation between farmyard manured and unmanured plots of the two fields, Barnfield and Woburn, using 10 gm. of soil as inoculum and the results are given in Tables 3 and 4.

Table 3. *Nitrogen fixation (in mg.) in 50 c.cm. mannitol solution inoculated with 10 gm. of soil.*

(Barnfield)

Experiment	Farmyard manured				Unmanured			
	Initial nitrogen	Fixation in days			Initial nitrogen	Fixation in days		
		10	20	30		10	20	30
1.	20.31	5.22	5.92	6.49	8.74	3.23	3.10	3.54
2.	20.74	6.34	7.05	7.76	9.73	1.83	2.11	2.82
3.	16.93	6.21	7.02	7.48	8.32	2.62	2.96	4.01
Average	19.52	5.92	6.63	7.24	8.93	2.56	2.72	3.45

Table 4. *Nitrogen fixation (in mg.) in 50 c.cm. mannitol solution inoculated with 10 gm. of soil*

(Woburn)

Experiment	Farmyard manured				Unmanured			
	Initial nitrogen	Fixation in days			Initial nitrogen	Fixation in days		
		10	20	30		10	20	30
1.	12.41	2.39	3.38	3.90	8.45	1.12	2.28	2.68
2.	11.99	1.12	3.50	4.00	8.04	2.39	2.70	3.02
3.	11.85	2.39	3.52	4.23	8.18	2.39	2.25	2.41
4.	11.85	2.95	3.87	3.72	7.75	1.83	2.65	2.90
Average	12.02	2.21	3.56	3.96	8.10	1.93	2.47	2.75

It may be observed that in both the cases the nitrogen fixation is comparatively more in manured than the corresponding unmanured plots and the difference is greater at Barnfield than at Woburn. The nitrogen fixation in the manured plot at Woburn again is slightly more than the unmanured plot at Barnfield although the nitrogen fixing flora in the latter is *Azotobacter* which is known to be superior in respect of nitrogen

fixation (Lipman and Burgess, 1915) than *Clostridium* present in Woburn soil.

(*ii*) *The influence of the quantity of inoculum on Nitrogen fixation*

The effect of variation in the quantity of inoculum on nitrogen fixation was examined by using 1–10 gm. of soil from both the plots of the two fields and the results are given in Table 5.

Table 5. *Nitrogen fixation (in mg.) in presence of different quantities of inoculum*

Quantity of inoculum		Farmyard manured			Unmanured			
		10 gm.	2 gm.	1 gm.	10 gm.	5 gm.	2 gm.	1 gm.
Woburn	Initial Nitrogen	12.22	2.37	1.26	8.11	3.95	1.55	0.79
	Fixation	3.91	3.27	3.01	2.82	2.96	3.00	3.01
Barnfield	Initial Nitrogen	19.32	—	1.97	8.93	—	—	0.84
	Fixation	7.24	—	5.55	3.45	—	—	5.40

It may be observed that when 1 gm. of soil from differently treated plots of the same field is used as inoculum, the amount of fixation is identical, although the plots may differ considerably in the degree of their fertility (Table 2). With a gradual increase in the inoculum, the nitrogen fixation rises in the farmyard manured plots, but shows a regular decrease in the unmanured ones. This dissimilar behaviour between the manured and unmanured plots of the two fields in presence of larger quantity of inoculum may be due to the physico-chemical character of a soil, or its microbial flora. The following experiment was accordingly carried out to examine the effect of eliminating the biological factor.

Varying quantities of soil, from the differently treated plots of a number of fields, were sterilized, each in a 250 c.cm. flask, and 50 c.cm. of sterile media was added to each of the flasks. They were then inoculated, each with 1 c.cm. soil suspension containing 0.01 gm. of soil from Barnfield farmyard manured plot. The results of nitrogen fixation are given in Table 6.

In all cases, the amount of fixation is fairly uniform, the quantity of sterile soil, or the difference in its physical or chemical nature, does not appear to have any effect. Further, the fixation in the control flasks where no sterile soil was present, was the same as in the experimental ones. The biological nature of the soil thus appears to play the deciding role. It has been established that nitrogen fixation by *Azotobacter* is considerably increased in the presence, not only of other bacteria (Mahmoud 1932), but also of different soil micro-organisms, including Algae (Jone 1930), Mycorrhiza-fungi (Waksman 1932) and Protozoa (Nasir 1923). Further experiments appear to confirm the above observations.

Table 6. *Effect of soil on nitrogen fixation (in mg.)*

	Broadbalk		Hoosfield		Barnfield		Barnfield				Woburn	Without Soil
	FYM	X	FYM	X	FYM	X	FYM	FYM	X	X	FYM	—
Quantity of inoculum	1 gm.	1 gm.	1 gm.	1 gm.	10 gm.	10 gm.	10 gm.	1 gm.	10 gm.	1 gm.	10 gm.	0.01
Initial Nitrogen	2.11	1.05	2.25	1.19	18.23	7.05	17.93	1.76	11.28	1.12	21.28	0.017
Fixation (A)	5.78	5.57	6.21	5.99	7.48	5.23	7.63	5.33	4.95	5.25	4.1	5.38
Fixation (B)	5.5	5.5	5.51	5.85	5.64	5.52	5.41	5.32	5.51	5.3	5.39	

Fixation (A) = Nitrogen fixation in presence of sterilized soils.

Fixation (B) = Nitrogen fixation after inoculation with Branfield soil.

FYM = Farmyard manured X = Unmanured

(iii) Effect of season on nitrogen fixation

Microbial population as well as their activity is known to vary considerably during different seasons (Cutler *et al.*, 1922, Fritsch 1913, Herdman 1922) and similar behaviour is expected in respect of nitrogen fixation in soil. The preceding experiments were carried out during the summer, and in order to investigate the effect of change of season on nitrogen fixation, experiments were repeated from December to March, and the results are given in Table 7.

Table 7. *Effect of Winter Season on Nitrogen fixation (in mg.)*

Inoculum		Broadbalk		Hoosfield		Barnfield	
		FYM	X	FYM	X	FYM	X
10 gm.		5.37	3.1	3.41	3.1	3.7	4.2
,, ,,		3.8	4.2	4.5	3.57	4.6	3.4
	Aver.	4.58	3.6	3.95	3.32	4.1	3.8
1 gm.		5.75	5.08	4.85	4.86	5.15	5.32
,, ,,		5.78	5.5	5.21	5.23	5.22	5.21
	Aver.	5.76	5.29	5.03	5.04	5.18	5.26

FYM = Farmyard manured X = Unmanured

It may be observed that the amount of fixation with 1 gm. of inoculum does not show any variation with the change of season, and there is no decrease or any significant difference between the differently treated plots of the same field. With 10 gm. of inoculum, however, the nitrogen fixations decreased decidedly even in the manured plots. Repeated experiments with soils from Barnfield, Broadbalk and Hoosfield confirmed this observation.

Further, in all flasks containing 1 gm. of soil there was a heavy growth of *Azotobacter* and the characteristic grey film developed on the culture solution. But in flasks inoculated with 10 gm. of soil the film as well as the culture solution were orange red, a colour usually associated with the nitrogen fixing group other than *Azotobacter*. The microscopic examination of the film and the solution showed the presence of *Steptrothrix* and numerous other slender bacteria while the number of *Azotobacter* were very few and they were observed forming a thin film on the wall of the flask just above the surface of the culture solution.

Discussion

Except for the use of nitrogen-free nutrient solution no standard procedure was laid down for the determination of nitrogen fixation in soil. The amount of nutrient solution, the quantity of soil used and even the period of incubation varied considerably with the different workers. In view of the diversity of experimental conditions it is not feasible to draw a satisfactory conclusion from a comparative study of these data.

In the present investigation it may be observed that variation nitrogen fixation between two plots of unequal productivity is reflected when 10 g. of soil is used as inoculum. They also indicate variation in nitrogen fixation with the change of season, while the fixation with a small quantity of inoculum is identical irrespective of soil productivity or change in season.

The differential behaviour with variation in the quantity of inoculum may be explained as follows. In a soil the different groups of micro-organisms are in a state of unstable equilibrium with a number of associative and antagonistive influences at work (Waksman 1932). When a small quantity of soil is used as inoculum the initial concentration of the different groups of micro-organisms is low, and as there is no source of nitrogen other than that from the inoculum itself, the growth and activity of the heterotropic non-nitrogen fixing bacteria is considerably restricted. The nitrogen fixing flora, on the other hand, multiply raipidly and attains a large population. It is well known that in a mixed bacterial culture, the high initial concentration of a species checks considerably the growth and activity of the rest. The nitrogen fixing flora thus acts more or less as a pure culture and at any time under similar condition would show very little variation in their nitrogen fixing capacity. With a definite quantity of favourable food, the same strain will fix an identical amount of nitrogen.

This explains the uniformity of nitrogen fixation with 1 gm. of inoculum, in two differently treated plots of the same field. Further, when 0.01 gm. of inoculum from Barnfield farmyard manured plot was used in the presence of different quantities of sterile soil, of diverse physical and chemical character, the amount of fixation was not only uniform, but was also equivalent to the amount fixed by 1 gm. Barnfield soil. The fixation by a small inoculum, therefore, indicates the nitrogen fixing capacity of the strain in soil and not of the soil itself.

The condition is different when a large inoculum is used. The initial concentration of other bacterial species is high, further as there is considerable amount of nitrogen in the inoculum itself, along with the growth of *Azotobacter*, there is also the development and activity of other miçro-organisms. The flora in the solution is no longer more or less exclusively of the nitrogen fixing type, but a composite group, varying both in composition and activity during the different periods in the season. The nitrogen fixation, therefore, is not constant as with a small inoculum, it may vary in either direction according to whether at any period the mixed flora is favourable or unfavourable to the process of nitrogen fixation. The result of nitrogen fixation during winter is also in agreement with and confirms the above conclusion.

Acknowledgement

The writer is indebted to Sir E. I. Russel, the Director, Rothamsted Experimental Station, Harpenden and to Dr. H. H. Mann, Director, Woburn Experimental station for all the necessary facilities. He also wishes to acknowledge the help and advice of Mr. D. W. Cutler, late of Rothamsted, in whose department the work was carried out.

References

Anon. (1945). Methods of Testing Biological Deterioration of Service Materials. *Office of Scientific Research and Development Report,* No. 6065, P. 24.

Brown, P. E. (1915). Bacterial activity and crop production. *Iowa Agr. Exp. Sta. Res. Bull.*, **25**.

Cutler, D. W., Crump, L. M. and Sandon, H. (1922). A quantitative Investigation of the Bacterial and Protozoan population of the Soil, with an account of the Protozoan Fauna. *Proc. Roy. Soc. Ser. B.*, **211**, 317–350.

Fritsch, F. E. and Rich, F. (1913). A four years' observation of a Fresh Water Pond. *Annales de Biologie Lacustre,* **6**.

Given, G. C., Kuhlman, G. and Kern, C. A. (1916-17). Velocity of non-symbiotic nitrogen fixation in soils of general fertilizer plot. *Pa. agric. Exp. Sta. Ann. Rpt.*, 405–409.

Herdman, W. A. (1922). Spolia Runiana V. *J. Linn. Soc.*, **46**, 141-170.

Jones, J. (1930). The investigation into the Bacterial Associations of some cyanophyceae with special reference to their nitrogen supply. *Ann. Bot. Lond.*, **44**, 721–740.

Mahmoud, S. (1932). A study of nitrogen fixing bacteria from soil. *Proc. Soc. Int. Con. Soil Sci.*, **86**.

Nasir, S. M. (1923). Some preliminary investigations on the relationship of Protozoa to soil fertility with special reference to nitrogen fixation. *Ann. Appl. Biol.*, **13**, 516–534.

Lipman, J. G., Burgess, P. S. (1915). Studies on nitrogen fixation and *Azotobacter* forms in soils of foreign countries. *Cent. Bakt,* II, Bd. **35**, 481–511.

Walton, J. H. (1915). *Azotobacter* and Nitrogen fixation in Indian soil. *Mem. Dept. of Agri. Ind. Bact. Ser.*

Waksman, S. A., Karunakar, P. D. (1924). Nitrogen fixation and mannite decomposition. *Soil. Sci.*, **17**, 384.

Waksman, S. A. (1932). *Principles of Soil Microbiology.* The William and Wilkins Company, Baltimore.

(*Received for publication 12 December 1956*)

Ind, J. mycol. Res., **2** (1 & 2), 37—40 (1956).

A SURVEY ON THE PRODUCTION OF VITAMIN B_{12} BY SOME ANTAGONISTIC STRAINS OF *STREPTOMYCES*

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The production of vitamin B_{12} by some antagonistic strains of *Streptomyces* sp. isolated from the soils of West Bengal, has been surveyed. Of the strains, 48% showed positive activity for vitamin B_{12}. When tested for antagonistic action, 64% of the strains indicated antibiotic activity against *Staphylococcus aureus*. Strains related to *S. fradiae, S. californicus* and 8–80 group were found to elaborate varying amounts of vitamin B_{12} as well as antibiotic substances. It was also observed that there was no apparent relationship between the production of vitamin B_{12} and that of antibiotic substances.

Introduction

The recent report about the ability of the strains of *Streptomyces chromogenes, S. griseus* and *S. antibioticus* (Rickes *et al.*, 1948) to synthesise vitamin B_{12} has stimulated further interest in the genus *Streptomyces*. A number of surveys undertaken in different laboratories have already reported the production of vitamin B_{12} by several species of *Streptomyces* (Darken, 1953). Some of the strains belonging to this group of micro-organisms, isolated in this laboratory mainly as a part of general survey of the microbial flora of Indian soils specially with regard to the production of antibiotic substances were simultaneously screened for the production of vitamin B_{12} (Mukherjee *et al.*, 1953). The present investigation was undertaken to find out whether micro-organisms producing antibiotic substances were to produce vitamin B_{12} as well, or whether the synthesis of vitamin B_{12} had any effect on the yield of antibiotic substances. Of special interest would be the determination of relationship, if there be any, between the production of antibiotic substance and that of a growth factor by a particular group of micro-organisms.

Materials and Methods

The strains belonging to *Streptomyces* spp. were maintained on potato-dextrose agar (Kelner, 1947). They were grown in a modified straw-infusion medium (Dey, 1947) in 250 ml. Erlenmeyer flasks for 8 days at 28°C. To obviate the introduction of methionine, $(NH_4)_2HPO_4$ (0.54%) was used as N-source instead of beef-extract (Lemco).

Prior to inoculation, sterile $CoCl_2$, H_2O solution (2 ppm.) was added to the medium. Spore-suspension (10 parts by volume) of the strains of *Streptomyces* in sterile water was added as inoculum. After incubation,

the fermented product was adjusted to *p*H 5.0, autoclaved at 115°C for 10 minutes, filtered and diluted to original volume by means of 1% phosphate buffer. 10 ml. of the test sample were treated with 0.8 mg. of KCN at 60°C. for one hour and assayed for total activity. The excess of cyanide was removed by aeration for 30 minutes.

The potency of the samples was next determined according to the method of Harrison and his co-workers (1951) using a strain of *Escherichia coli* mutant (NCIB 8134) as test organism. The ease of growing the bacteria in a comparatively simple medium together with its specificity for B_{12} prompted us to select *E. coli* mutant as a test strain for assay. It was reported that the mutant utilised methionine in addition to B_{12} for growth. It was also observed by Chiao and Peterson (1953) that when the ratio of methionine to B_{12} on a weight basis was 50,000 or less, the presence of the former did not introduce any significant error in the assay. Since $(NH_4)_2HPO_4$ was used as N-source, it was thought that methionine, even if synthesised or produced as a result of autolysis, would not be present in amounts sufficient to cause interference with the assay.

A standard curve was drawn using crystalline vitamin B_{12} (Glaxo) and the activity of samples was determined by extrapolation when required. The antagonistic action of *Streptomyces* spp. was determined against *S. aureus* by agar cup method of assay.

Tests were simultaneously run to check the production of methionine as a metabolite by means of one-dimensional paper-chromatography using phenol-water as a developing solvent.

Results

Of the strains tested for the production of vitamin B_{12}, twenty-four (48%) showed positive activity. Of them, as many as fourteen (28%) strains, produced more than 0.08 μgm./l. When tested by paper-chromatography, methionine was not found to be present at least in detectable amount.

On the other hand when tested for antagonistic action 32 (64%) strains were found to possess positive activity against *S. aureus*.

Table 1 shows the production of vitamin B_{12} by strains of *Streptomyces* along with their antagonistic action against *S. aureus*. The diameter of the zone of inhibition in each case was recorded to the nearest mm.

Of the isolates, strains belonging to *S. fradiae*, *S. californicus* and 8–80 group, characterised according to Pridham and Gottlieb (1948), and marked with asterisks in Table 1, were found to elaborate varying amounts of vitamin B_{12} under identical conditions of fermentation.

Discussion

The present investigation confirms the previous observation that the species of *Streptomyces* can produce vitamin B_{12}, when grown on media containing cobalt salts (Darken, 1953). Strains related to a particular group of *Streptomyces* spp., were found to elaborate varying amounts of B_{12} as well as antibiotic substances (Table 1). It was also observed that

Table 1. *Production of vitamin B_{12} and the antagonistic activity of different strains of* Streptomyces *spp.*

Isolate No.	Identity	Average yield of vitamin B_{12} (μgm. /l)	Antagonistic activity against *S. aureus* as measured by zone of inhibition (mm.)
Ac_7 (8)	*S. erythrochromogenes*	20	23.0
Ac_3 (9)	,,	-	23.5
Ac_1 (34)	,,	70	24.0
Ac_3 (177)	,,		19.5
Ac_{14} (193)	,,	—	14.0
Ac_{X2} (9)	*S. albosporeus*	—	20.0
Ac_3 (195)	,,	—	20.0
*Ac_4 (10)	*S. californicus*	150	13.0
*Ac_4 (198)	,,	210	—
*Ac_1 (218)	,,	—	15.0
Ac_{10} (193)	*S. alboflavus*	70	20.0
Ac_1 (27)	*Streptomyces* sp. related to *S. alboflavus.*	160	—
Ac_1 (214)	*S. viridochromogenes*	20	—
Ac_{X1} (32)	*Streptomyces* sp. related to *S. viridochromogenes.*	170	—
*Ac_1 (46)	*S. fradiae* (mutant ?)	60	14.0
Ac_2 (51)	*Streptomyces* sp. related to *S. griseus.*	250	—
Ac_6 (125)	,,	280	—
Ac_6 (177)	,,	—	10.0
Ac_1 (64)	*S. flavovirens*	—	—
Ac_1 (67)	*S. erythreus*	10	15.0
Ac_2 (95)	*S. cellulosae*	—	16.0
Ac_3 (185)	*S. halstedii*	—	29.5
Ac_4 (195)		10	29.5
Ac_{12} (B1)		—	28.0
Ac_6 (118)	*Streptomyces* sp. related to *S. halstedii.*	—	23.0
Ac_1 (82)	*Streptomyces* sp. related to *S. albus*	140	—
Ac_3 (118)	,,	90	—
Ac_2 (177)	*S. rutgersensis*	—	25.0
Ac_3 (183)	,,	- -	23.0
Ac_2 (197)	,,	—	25.0
Ac_3 (177)	*S. albidoflavus*	120	—
Ac_2 (185)	*Streptomyces* sp. related to *S. albidoflavus.*	90	16.0
Ac_3 (194)	*S. griseolus*	180	—
Ac_{11} (195)	*Streptomyces* sp. related to *S. scabies.*	—	24.0
Ac_3 (196)	*S. lavendulae* (mutant ?)		—
Ac_{12} (217)	*S. purpeochromogenes*	-	12.0
Ac_8 (185)	*Streptomyces* sp. related to *S. flaveolus.*	—	23.0
*Ac_3 (203)	*Streptomyces* sp. related to *S. fradiae* and *S. californicus.*	70	24.0
Ac_1 (9)	*Streptomyces* sp. (*Unidentified*)	80	—
Ac_1 (60)	,,	—	14.0
Ac_3 (BG)	,,	- -	15.0
Ac_9 (177)	,,	120	—
Ac_4 (193)	,,	150	-
Ac_6 (193)	,,	140	-
Ac_{X3} (194)	,,	- -	30.0
Ac_4 (203)	,,	20	24.0
Ac_6 (217)	,,	—	—
Ac_4 (B1)	,,	- -	23.0
Ac_5 (B1)	,,	—	20.0

several strains which did not produce antibiotic substance were able to synthesise vitamin B_{12}. The reverse was also true. The synthesis of these complex organic metabolites, one growth inhibiting and the other growth accessory, by a group of micro-organisms, often a single species, presents a fascinating problem. From the preliminary observations presented here, it appears that the production of B_{12} does not bear even a qualitative relationship with that of antibiotic substances. It has been found that there is a much closer correlation between growth and the production of B_{12} than there is between growth and the production of the antibiotic substance (Dulaney and Williams, 1953). On the other hand, the porphyrin-like nature of grisein—an antibiotic substance produced by a strain of *S. griseus* a similar nature of a part of the B_{12} molecule, the observation that the most probable action of stimulators for the production of B_{12} lies in their being competitive inhibitors (Dulaney and Williams, 1953), the use of some of the antibiotics as growth stimulators in the form of food-supplement—all these suggest some relationship between production of vitamin B_{12} and that of antibiotic substance by a perticular genus. It is of interest that in a conjunctive fermentation. the trend of the curve of vitamin B_{12} production was found to be parallel to that of the production of the antibiotic substance throughout the fermentation cycle (Buchanan *et al.*, 1950). No final word can, however, be said at the present state of our knowledge, until the mechanism of biosynthesis of these complex molecules is clearly understood.

Acknowledgment

The authors are greatly indebted to Dr. D. M. Bose, Director, Bose Research Institute, for allowing them to carry out the work in the laboratory of the Department of Microbiology. They are also thankful to Dr. P. N. Nandi, Head of the Department of Microbiology, Bose Research Institute, for his interest in the work.

References

Buchanan, J. G., Johnson, A. W., Mills, J. A. and Todd, A. R. (1950). Isolation of phosphorus containing degradation products from vitamins B_{12} and $B_{12}C$. *Chem. and Ind.*, **22**, 426.

Chiao, J. S. and Peterson, W. H. (1953). Microbiological assay of vitamin B_{12} with a mutant of *Escherichia coli*. *Appl. Microbiol.*, **1**, 42–46.

Darken, M. A. (1953). Production of vitamin B_{12} by micro-organisms and its occurrence in plant tissues. *Bot. Rev.*, **19**, 99–130.

Dey, N. C. (1947). On the production of Streptomycin. I. Selection of media. *Sci. and Cult.*, **12**, 100–103.

Dulaney, E. L. and Williams, P. L. (1953). Observations on *Streptomyces griseus*. V. Synthetic media for vitamin B_{12} production with special reference to possible vitamin B_{12} precursors. *Mycologia*, **45**, 345–358.

Harrison, E., Lees, K. A. and Wood, F. (1951). Assay of vitamin B_{12}. VI. Microbiological estimation with a mutant of *Escherichia coli* by the plate method. *Analyst*, **76**, 696.

Kelner, A. and Morton, H. E. (1947). Two antibiotics (lavendulin and actinorubin) produced by actinomycetes. *J. Bact.*, **53**, 695–704.

Mukherjee, S. K., Sen, G. P. and Nandi, P. N. (1954). Production of vitamn B_{12} by some *Streptomyces* spp. *Proc. 41st Ind. Sci. Cong.*, Pt. III, 129.

Pridham, T. G. and Gottlieb, D. (1948). The utilisation of carbon compounds by some actinomycetales as an aid for species determination. *J. Bact.*, **56**, 107–114.

Rickes, E. L., Bring, N. G., Koniuszy, F. R., Wood, T. R. and Folkers, K. (1948). Comparative data on vitamin B_{12} from liver and from a new source, *Streptomyces griseus*. *Science*, **106**, 634–636.

(*Received for publication 5 May 1957*)

Ind. J. mycol. Res., **2** (1 & 2), 41–50 (1956).

CONTRIBUTIONS TO THE CYTOLOGY OF HYMENOMYCETES :

II. KARYOLOGICAL OBSERVATIONS IN *STEREUM FUSCUM* (SCHRAD.) QUÉL.

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(With 2 Text-figures)

This investigation was undertaken in 1954 with a view to study the nuclear behaviour in the life-cycle of *Stereum fuscum*, a very common species of Thelephoraceae occurring as a saprophyte on logs and posts of *Shorea robusta* in West Bengal, during the wet months of July to September.

It has been found that its karyological features are consistent with its sexually 'heterothallic' and 'bipolar' nature.

The typical dikaryon in the young basidium fuses to form a large fusion-nucleus in which reticulate structures of chromatin materials appear in the early stages of meiosis. The reticulum condenses greatly during the subsequent stages in which there appear chromosome-like structures and ultimately, three chromosomes constituting the haploid complement seggregate into each of the two daughter nuclei formed at the poles of a transverse spindle. Two subsequent divisions in each of them eventually produce eight daughter nuclei within the tetra-sterigmatic basidium on which are developed four basidiospores. Each of the spores receives a single basidial nucleus and the remaining four nuclei degenerate within the basidium which subsequently becomes empty after the spores have been shed.

Mature basidiospores are always uninucleate. On germination, the nucleus in each divides and one daughter nucleus passes into the germ-tube. This nucleus divides further and its descendants eventually produce a four to five-nucleate hypha which is at first aseptate and sparsely branched. In later stages, only the tip-portion of this hypha becomes septate and is resolved into typical monokaryotic cells while its distal part remains aseptate and multinucleate.

The primary mycelium developing from a single spore always lacks clamp-connections and is in all cases composed of uninucleate cells. The secondary mycelium, on the other hand, shows abundant clamp-connections due to conjugate division of the heterodikaryon in each cell. Cytoplasmic chromatic granules, well distinguishable from the nuclei, are present in all the afore-said stages in the life-cycle of the fungus.

Introduction

From earlier times efforts were being made by workers like Strasburger (1884), Rosenvinge (1886), Wager (1891–1899) and Dangeard (1895) to reveal the karyological phenomena in the life-cycle of Hymenomycetes and the latter worker reported nuclear fusion in the basidia of *Polyporus versicolor* (L.) Fr. Our knowledge in the cytology of Hymenomycetes

made considerable progress due to subsequent researches of Maire (1902), Lévine (1913), Kniep (1913–1916), Juel (1916), Sass (1928-1935), Wakayama (1930, 1932) and others. Chromosome numbers of a number of Hymenomycetes were also recorded. Since then, investigations on fungal cytology as applied to Hymenomycetes have been steadily increasing in number as will be evident from Olive's (1953) critical review on the subject. In so far as Indian works on Hymenomycetes are concerned, it was Bose (1937) who first described the basidial cytology of eleven species of polypores and four species of agarics with special reference to some extranuclear protoplasmic elements. Cytological studies on several Hymenomycetes occurring in India have recently been undertaken in this laboratory and the first contribution of the series was made by Banerjee and Mukherjee (1955).

In recent years there has been a growing tendency to look for a cytological basis which underlies the sexual reproduction in fungi. Considering the broad use of the term heterothallism as applied to fungi, Whitehouse (1949) has expressed his judgement that the essential criteria of sexual reproduction are nuclear fusion and meiosis in the life-cycle irrespective of the presence or not of differentiated sex organs or gametangia. This is particularly suggestive in the case of Hymenomycetes where sex organs are not differentiated and the great majority of which are heterothallic with various means by which the monokaryophasic mycelia are diploidized. The compatible monokaryophasic mycelia are superficially indistinguishable from each other and it will be evident from Olive's (1953) review that instances are not uncommon in Hymenomycetes where clamp-connexions are absent in the hyphae and yet the cells composing them are not monokaryotic. Incidentally, it has been observed by the writers in course of their studies on the biology of *Stereum fuscum* (Schrad.) Quél. a common species of Thelephoraceae occurring in India, that the fungus in question is 'heterothallic' like the majority of Hymenomycetes and is sexually 'bipolar'. But as far as informations are available, karyological conditions in *Stereum fuscum* have not been studied. The object of the present investigation was, on the one hand to study in general the cytology of *Stereum fuscum*, and on the other to examine its sexuality in the light of the nuclear phenomena in the life-cycle of the species. In the following pages will be described the karyological conditions of the spores, germinating spores, primary and secondary mycelia as well as the cytology of the basidia of *Stereum fuscum*.

Materials and Methods

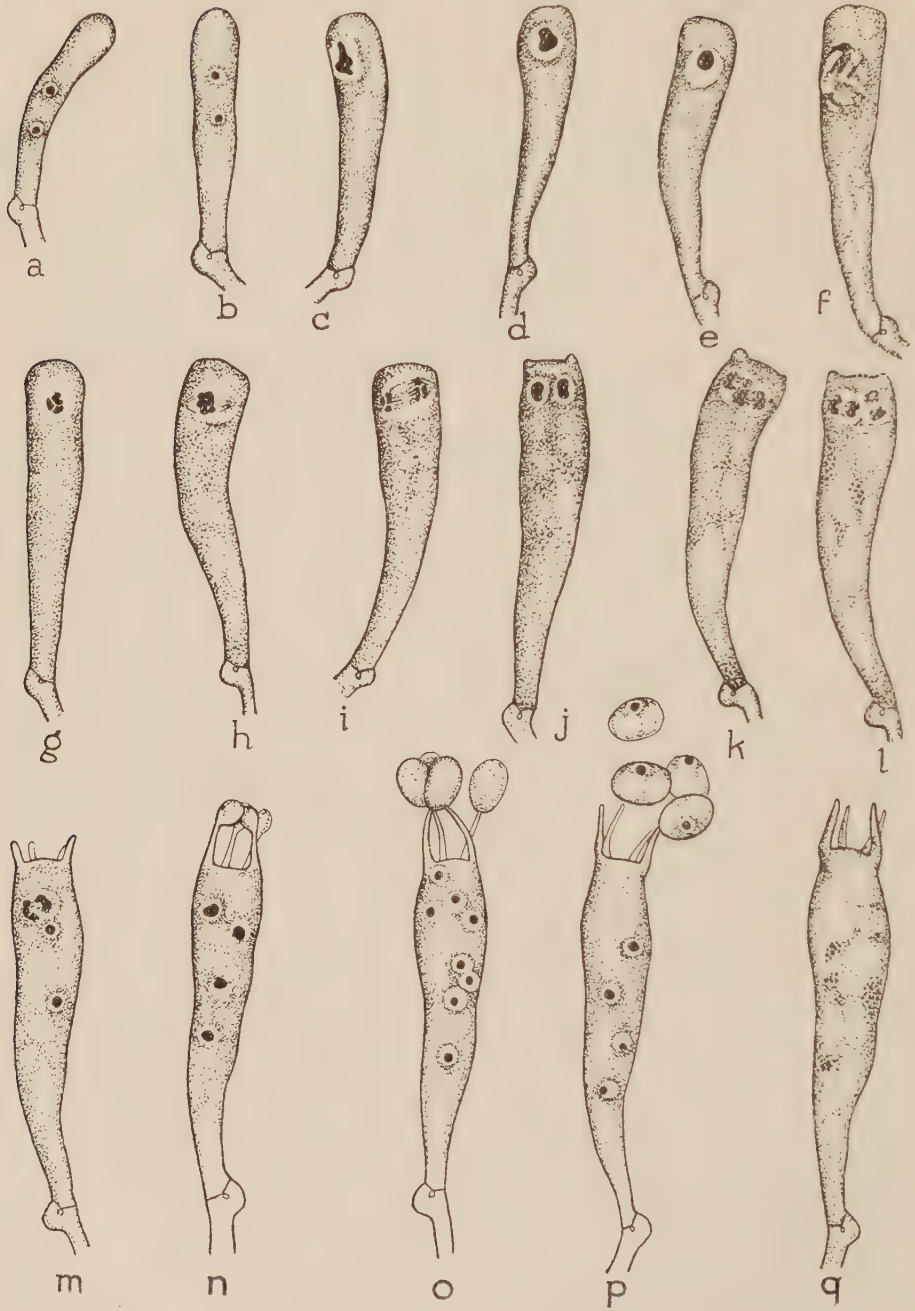
For studying nuclear conditions within the basidium, fresh fruit-bodies of *Stereum fuscum* were collected from Calcutta, after a brief shower, and immediately fixed at different intervals of time, *viz.*, at 11 a.m., 12.30 p.m. and 2 p.m. Of the fixatives used were 'Bouin-Allen', 'Formal-Acetic-Alcohol', 'Navashin (A & B)', 'Sass' and 'La Cour (2BD)'. The results of staining varied with the nature of the fixative and also with the stain used. 'Bouin-Allen' and 'Sass' proved to be the most satisfactory fixatives

for general staining purposes. After fixation, the materials were washed, dehydrated and embedded in paraffin in the usual way following alcohol-chloroform-paraffin schedule. Microtome sections were cut at 6–12 μ thicknesses and by trial it was found that sections 6–8 μ thick were the most suitable ones for studying the basidial cytology of this species. Difficulties, however, were encountered with regard to the selection of proper stains and the staining procedure following proper fixation. After preliminary trials with several stains such as Heidenhein's Iron-Haematoxylin, aquous Basic Fuchsine (Feulgen's method, 1924) and aquous Crystal Violet, the first one could bring about the most satisfactory result when used after fixation with 'Bouin-Allen' or 'Sass'. It was noted that the best preparations may be obtained by staining the materials in Iron-Haematoxylin for two hours at 40°C. following mordanting in 4% Iron-Alum for one hour and a half.

Kniep's (1913) agar-film technique and that modified by Sass (1929) were tried for making total preparations in order to study the nuclear phenomena in the spores, germinating spores and well-developed mycelia, both primary and secondary. Spores from a fresh fruit-body were allowed to fall directly on a thin film of sterilized clear agar made on a slide, taking extreme aseptic measures to avoid chances of contamination. They were immediately fixed in several fixatives in order to observe the karyological conditions within them. For the study of nuclei in the germinating spores, spore-deposits on agar-films were incubated and these served as the materials when fixed at the desired stages of germination. Spores and germinated spores were best fixed with 'Sass' and stained in Iron-Haematoxylin. Total preparations of primary and secondary mycelia were also obtained in the same way by inoculating sterile agar-films with the respective inocula. All the preparations of germinating spores and those of primary and secondary mycelia were stained satisfactorily with Iron-Haematoxylin by following the same procedure as done in the case of the basidia.

Observations

Observations on the karyology of the species under consideration may well begin with the study of the basidia. They are more or less clavate, tetra-sterigmatic and quadrisporous, each with a clamp-connexion at the base. Young basidia appear as considerably swollen terminal hyphal cells and they are distinctly binucleate (Text-fig. 1, a & b), one nucleus lying a little distance above the other. The nucleoli are deeply stained with Heidenhein's Iron-Haematoxylin, leaving a more or less hyaline zone of karyolymph around each and this is specially prominent in the fusion-nucleus (Text-fig. 1, c–e). Following Pinto-lopes (1949), this type of nucleus may be called *compact and homogeneous*. According to this author, the nucleus when presents a homogeneous aspect, it becomes small, its chromatin and nucleolar-matter are contracted in an intensely stainable homogeneous body which is feulgen-positive and the major part of the nucleoplasm is free from the chromatin. In the *disperse-expanded* type according to him, on the other hand, diffused chromatin and chromocentres are to be found. The binucleate condition of the basidium is



Text-fig. 1. Karyological phenomena in the basidia of *Stereum fuscum*. × 3000.

(For explanation, vide text).

maintained until it attains a fairly clavate form after which karyogamy occurs. During karyogamy, the two nucleoli with their respective chromatin-matter fuse to form a large deep-staining body within the more or less hyaline nucleoplasm (Text-fig. 1, c–e). The synkaryon then moves towards the upper part of the basidium. Subsequently, the fusion-nucleus presents a distinct reticulate structure, somewhat similar to that reported by Wakayama (1930, 1932) in a number of Hymenomycetes. The fusion-nucleus eventually takes up a very characteristic appearance (Text-fig. 1, f). The reticulum condenses greatly and the strands begin to thicken, as if they are going to be resolved into individual chromosomes. The thickening and condensation, however, are not uniform; but ultimately distinct heterochromatic bodies are produced by the condensing reticulum (Text-fig. 1, f). These heterochromatic bodies are provided with 'chromatic tails' which gradually fade away after a short distance. Such heteropycnotic chromatic bodies are observed only in the first meiotic prophase and much simulate chromosomes on which the allocyclic heterochromatin appears more condensed. According to Olive (1953), these heterochromatic bodies observed on the chromosomes of meiotic prophase and the so-called 'protochromosomes' of certain authors are probably homologous with the chromocenters which have been demonstrated by Heim (1951a, 1951b, 1952) in the vegetative nuclei of a large number of Ascomycetes. Various stages of heteropycnosis may be observed during the early meiotic prophase and it may be concluded that this is a long process relative to other stages of nuclear division. Exact count of the so-called *protochromosomes*, can not be taken. With chance of inaccuracy, due to close mingling, their number comes out to be 6–8 (Text-fig. 1, f). The haploid chromosome number has been verified in all cases to be 3 by counts from the first meiotic metaphase at which 3 closely associated bivalents may be seen in the polar and side views (Text-fig. 1, g & h). Later on, the 3 bivalents separate at anaphase-I, three chromosomes constituting a genome going to each of the two poles of a transverse spindle (Text-fig. 1, i). After completion of the first meiotic division, the resulting two nuclei remain side by side in the terminal portion of the basidium (Text-fig. 1, j). At this stage, the basidia have nearly reached their full dimension and show rudiments of sterigmata. A further division of both the nuclei then follows. These divisions are synchronous in most of the cases (Text-fig. 1, k & l), but rarely the division of one of the two nuclei is little belated (Text-fig. 1, m). Orientation of spindles in these divisions is variable but at least one of the spindles appears to be transverse. The other has been found to be transverse, obliquely transverse or nearly longitudinal and one usually remains a little above the other. These findings may be a strong support to the current interpretation that the types of spindle-orientation in Basidiomycetes are due to spacial reasons and necessarily do not always bear absolute phylogenetic and taxonomic value. By the time the second meiotic division is complete, four sterigmata appear and the four nuclei may be seen within the basidium without any definite arrangement. A third division in each of these four nuclei gives rise to the 8-nucleate stage of the basidium (Text-fig. 1, o). Meanwhile, four basidiospores are developed on four sterigmata and each of them receives

a single nucleus from the basidium, which still retains the remaining four nuclei within it (Text-fig. 1, p). The fate of these four supernumerary nuclei has not been studied in detail but, undoubtedly they do not enter into spores. In all probability, the four extra nuclei degenerate within the basidium which eventually becomes empty after the spores have been shed (Text-fig. 1, q). In several cases, basidia have been found to be collapsing even before the retained nuclei within them have fully degenerated.

The mature spore has always been found to contain a single nucleus within it (Text-fig. 2, a–d). The nucleolus and the chromatin are condensed into a central deep-staining body with a hyaline zone around it. Adjacent to the nucleus there are one or two vacuoles in the surrounding cytoplasm and these appear hyaline in the preparations (Text-fig. 2, a–d). When germination starts, the nucleus slightly enlarges, its chromatic material becomes more deep-staining but the reticulum is absent. At first, a short germ-tube is produced from one end of the spore while the nucleus remains within the spore-case. A few spores, however, have also been found to produce two germ-tubes at opposite ends of the spore-wall (Text-fig. 2, r). As the germ-tube elongates a little, the nucleus moves up to the base of the germ-tube and the cytoplasm within the spore becomes distinctly vacuolate (Text-fig. 2, f–i). After the germ-tube has attained a considerable length, the nucleus divides into two and a daughter nucleus passes into the germ-tube (Text-fig. 2, j & k). Septum-formation is deferred for the time being and the nucleus within the germ-tube divides once again (Text-fig. 2, l–n). Division of the basal nucleus within the spore-case can also take place when two germ-tubes are produced at opposite ends. The first two nuclear divisions take place in rapid succession after which the germ-tube elongates considerably (Text-fig. 2, m–o) and further nuclear divisions are somewhat delayed. The germ-tube does not usually branch even after it has attained a considerable length. Occasionally, however, a branch may be found to develop from the lower part of the germ-tube (Text-fig. 2, o-p) and it eventually receives the nuclear supply from the nearest nucleus of the germ-tube. The resulting hypha ultimately becomes 4 to 5-nucleate after which septation begins only in its upper part, resolving the tip-portion of it into uninucleate cells while the lower part of the hypha still remains multinucleate (Text-fig. 2, p–r). No clamp-connexion has been observed in this primary mycelium arising from a single germinated basidiospore. Such a finding is in harmony with the observations that *Stereum fuscum* is heterothallic in its sexual behaviour and has clamp-connexions only in the secondary mycelia obtained through pairing of two compatible monosporous mycelia.

Primary mycelia developed from several single-spore-cultures and also belonging to opposite sexual strains, when examined, appear to have the same essential morphological and karyological characteristics and the cells composing them are always uninucleate (Text-fig. 2, s–u). Chromatic materials of variable shape and size are also abundantly present in the cytoplasm. These chromatic granules have also been observed in spores, secondary mycelia as well as in the basidia. They are readily distinguished from the chromatic body of the nucleus on account of their much smaller size and lack of any hyaline zone around them.

Text-fig. 2. Nuclear phenomena in basidiospores, germinating basidiospores and in mycelia (primary and secondary) of *Stereum fuscum*. × 750.

(For explanation, vide text).

The secondary mycelium of *Stereum fuscum* is nodose-septate and possesses clamp-connexions as a result of conjugate division of the two nuclei in each cell. The nuclei are of the same general structure as in the case of primary mycelia and spores but they are typically arranged in conjugate pairs in each cell (Text-fig. 2, v–z). Occasionally, a clamp-connexion has been found to proliferate into a branch, which in turn forms clamp-connexions immediately after its emergence (Text-fig. 2, x–y). The subsequent increase in diameter of the proliferating hypha may, ultimately, almost obliterate its nature of origin from a clamp-connexion of the parent hypha (Text-fig. 2, y).

Discussion

Nuclear phenomena in the life-history of *Stereum fuscum* agree fully with the nature of its sexuality in as much as it has been observed by the writers to be 'heterothallic' and 'bipolar'. The mature spores and the cells of the primary mycelium are always monokaryotic. Spores and primary mycelia of opposite sexual strains are morphologically identical and have nuclei of similar morphology and staining properties. Furthermore, clamp-connexions are entirely absent in such mycelia. The basidiospore germinates to form a short 'homokaryotic' multinucleate hypha with little or no branching. In later stages, only the tip-portion of the hypha becomes segmented into uninucleate cells from which develops the monokaryophasic mycelium. Haploid nuclei present a more or less generalized aspect in their structure with a central deep-staining nucleolar body around which lies a hyaline zone of karyolymph. Dikaryophasic hyphae always have clamp-connexions and the affinity between the nuclei of opposite sexual strains is illustrated by the conjugate association of 'heterodikaryons' within each cell of the secondary mycelium as well as within the initial of basidium. Karyogamy in the basidium represents the true sexual act and is followed by seggregation of parental nuclei, perhaps with some re-combinations, after the completion of meiosis. During the early stages of meiosis, the fusion-nucleus in the basidium becomes distinctly reticulated by the condensation of deep-staining chromatin matter and heterochromatic knots on chromosome-like bodies may be located in the later stages. A somewhat similar cytological aspect of the diploid nucleus in early meiotic prophase has already been reported by Wakayama (1930, 1932). To quote Olive (1953), "the chromocentres of the vegetative nuclei are probably homologous with heterochromatic bodies observed on the chromosomes at meiotic prophase and with the so-called 'protochromosomes' of certain authors". In *Stereum fuscum*, the diploid and haploid chromosome numbers have been found to be 6 and 3 respectively. The fusion-nucleus undergoes the usual heterotypic and homotypic divisions of meiosis resulting in the production of four nuclei. A third division in all these four nuclei, ultimately gives rise to an eight-nucleate tetra-sterigmatic basidium, on which are developed four basidiospores, each receiving a single nucleus from the basidium. The remaining

four nuclei within the basidium degenerate. The existence of three successive nuclear divisions within the basidium is nothing unfamiliar and has been reported previously in *Craterellus cornucopioides* (Juel, 1916; Wakayama, 1932), *Corticium rolfsii* (Goto, 1936), *Peniophora ludoviciana* (Biggs, 1938), *Sistotrema confluens* (Kühner, 1947) and in a number of other Hymenomycetes. Rogers' (1934) challenge on the validity of the type of orientation of spindles during meiosis as the primary criterion in maintaining chiastobasidial and stichobasidial series within the Basidiomycetes seems justified, in Homobasidiae at least, when the inconstancy in the orientation of spindles during the three successive nuclear divisions in the basidium of *Stereum fuscum* is taken into consideration. In this species, it has been observed that the spindle, during the first meiotic division is transverse; but in the second homotypic division, one of the spindles is transversely oriented but the other has been found to range in orientation from vertical to transverse or oblique. This may suggest that the pattern of spindle-orientation may be determined by space within the basidium and it tends to be transverse when the basidium is broad or becomes vertical when it is long and narrow.

References

Banerjee, S. N. and Mukherjee, N. (1955). Contributions to the cytology of Hymenomycetes. I. Cytological studies in *Marasmius campanella* Holterm. *Proc. nat. Inst. Sci. Ind.*, **21** (3), 115–119.

Biggs, R. (1938). Cultural studies in the Thelephoraceae and related fungi. *Mycologia*, **30**, 64–78.

Bose, S. R. (1937). Cytology-study of basidia of Polyporaceae. *Jour. Ind. Bot. Soc.*, **16** 119–128.

Dangeard, P. A. (1895). Mémoire sur la reproduction sexuelle des Basidiomycêtes. *Le Botaniste*, **4**, 119–181.

Feulgen, R. and Rossenbeck, H. (1924). Mikroskopish-chemischer Nachweis einer Nucleinsaure von Typus der Thymonuclein saure und die darauf beruhende elektive Farbung Vom Zellkrnen in mikroskopischen Praparaten. *Zts. Phys. Chem.*, **135**, 203–248. Cited by La Cour, L. in *Bot. Rev.*, **3** (5), 241–257.

Goto, K. (1936). *Sclerotium Rolfsii* Sacc. in perfect stage. IV. Cytological observations. *Ann. Phytopath. Soc. Japan*, **6**, 101–118.

Heim, M. P. (1951a). Sur la structure du noyan chez les Discomycêtes. *Compt. Rend. Acad. Sci.* (Paris), **232**, 1693–1695.

Heim, M.P. (1951b). Remarques sur les divisions nucléaire chez les Discomycêtes. *Campt. Rend. Acad. Sci.* (Paris), **232**, 2468–2470.

Heim, M.P. (1952). Observations sur le noyan des Ascomycetes. *Rev. de Mycol.*, **17**, 3–38.

Juel, H. O. (1916). Cytologische Pilzstudien. I. Die Basidien der Gattungen *Cantharellus*, *Craterellus* und *Clavaria*. *Nov. Acta. R. Soc. Sci. Upsal.*, Ser. IV, **4** (6).

Kniep. H. (1913). Beiträge zur Kenntnis der Hymenomyceten, I & II. *Zts. Bot.*, **5**, 593–637.

Kühner, R. (1947). Le comportement nucléaire dans les basides uniformes et à plus de quatres spores de *Sistotrema confluens* Pers. *Compt. Rend. Acad. Sci.* (Paris), **224**, 1068–1069.

Lévine, M. (1913). Studies in the cytology of Hymenomycetes, especially the Boleti. *Bull. Torrey, Bot. Cl.*, **40**, 137–181.

Maire, R. (1902). Researches cytologiques et taxonomiques sur les Basidiomycêtes. *Thèse Faculté Sciences*, Paris. Cited by Bose, S. R. in *Jour. Ind. Bot. Soc.*, 1937, **16**, 119–128.

Olive, L. S. (1953). The structure and behaviour of fungus nuclei. *Bot. Rev.*, **19**, 439–586.

Pinto-Lopes, J. (1949). Contribution to the study of the nuclear structure in fungi. *Separata de Portugalie Acta Biologica*, **2**, 191–210.

Rogers, D. P. (1934). The basidium. *Univ. Iowa Stud. Nat. Hist.*, **16**, 160–182.

Rosenvinge, L. K. (1886). Sur les noyaux des Hyménomycêtes. *Ann. Sci. Nat. Bot.*, **7** (3), 75–91.

Sass, J. E. (1929). The cytological basis for Homothallism and Heterothallism in the Agaricaceae. *Amer. Jour. Bot.*, **16**, 663–700.

Strasburger, E. (1884). *Das botanische Praktikum*, Jena.

Wager, H. (1891–1899). On the nuclei and nuclear division in Hymenomycetes. *Ann. Bot.* **5**, **6**, **7**, **8** and **13**.

Whitehouse, H. L. K. (1949). Heterothallism and sex in the fungi. *Biol. Rev.*, **24**, 411–447.

Wakayama, K. (1930). Contributions to the cytology of fungi. I. Chromosome number in the Agaricaceae. *Cytologia*, **1**, 369–388.

Wakayama, K. (1932). Contributions to the cytology of fungi. IV. Chromosome number in Autobasidiomycetes. *Cytologia*, **3**, 260–284.

(*Received for publication 8 February 1957*)

Ind. j. mycol. Res., **2** (1 & 2), 51—56 (1956).

ATTENUATION OF BEERWORT BY STRAINS OF *SACCHAROMYCES CEREVISIAE* HANS.

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With a view to select a strain of yeast which will be suitable for the manufacture of beer under local conditions, different strains of yeast, *Saccharomyces cerevisiae*, were obtained from different sources. These strains were plated out in beer wort-agar medium and pure cultures from single colonies were established. A comparison of the degree of attenuation amongst the different strains of yeast was made. It has been shown that a single strain of pure yeast is more suitable for beerwort fermentation than mixed races.

Introduction

The use of selected strains of *Saccharomyces cerevisiae* in fermenting beerwort has long been adopted in all breweries of the world. The selection of such strains is based principally upon the ability of the yeast in question to break down the constituents of the wort during fermentation. In order to study the activity of yeast strains on the medium of beerwort in which they are allowed to grow and ferment, the use of a wort of almost uniform composition is essential. The quality of the fermentation products depends upon the nature of the medium and the manner in which the yeast cells behave. Neither too fast nor too slow a growth will be desirable for producing a fermentation product presenting all the essential qualities.

The success of fermentation in breweries depends not only upon the amount of alcohol in the beer, but also in large measures upon its flavour, taste and stability. It is the aim of the brewer to conduct fermentation in such a controlled manner that by mixing a healthy type of pitching yeast with the beerwort, a rapid growth of yeast may be ensured, and the access of other harmful micro-organisms checked. It may be pointed out, however, that a small population of lactic acid bacteria (*Lactobacillus* sp.) is always present in a brew and help in the final quality of the bear. Different strains of yeast, whether mixed types or pure races, can be distinguished by their rate of fermentation or the degree to which they will attenuate under normal conditions of a particular fermentation system. The difference of attenuation* depends largely upon the flocculating properties** of yeasts. Some yeasts tend to flocculate rapidly in the wort,

* The term "attenuation" means the rate of utilisation of the food-constituents of the wort by the yeast cells.

** "Flocculating property" means the clumping power of the yeast cells either at the bottom or on the top of the fermenting liquid.

while others remain in suspension. The highly flocculating yeasts tend to attenuate the beerwort very slowly. Thus there is a reciprocal relation between flocculation and attenuation.

In breweries two methods of fermentation are adopted—(1) top fermentation, and (2) bottom fermentation. The former process is a faster one than the latter. The following experiments were, therefore, set up to ascertain the changes in the fermenting medium on inoculation with different selected strains of pitching yeast, as well as the attenuating power of these strains with a view to find out a strain of bottom fermenting yeast most suitable for the manufacture of beer under local conditions.

Materials and Methods

Pure cultures of *S. cerevisiae*, obtained from various sources, were periodically examined, and subcultures were maintained on unhopped beerwort-agar medium. Index numbers of the yeast strains were as follows:— M_5, B_1, B_2, B_3 and B_{10} .

Cooled beerwort was pumped into experimental vats (capacity 40 gallons each) and pitched with freshly-grown yeast cultures, taking care of the fact that the number of cells in the pitched wort did not exceed 3,000 cells per c.c. The vats were placed in the fermentation chamber fitted with cooling coils, and the temperature was maintained constantly at $50°\pm2°F$.

The samples of wort from the respective vats were collected and analysed before and after pitching, according to the schedule mentioned below. (1) The specific gravity was recorded at a constant temperature of 20°C: the amount of total solids was measured in terms of °Brix at the above temperature; (2) the reducing sugar was estimated with Fehling's solutions and expressed in terms of maltose; and (3) the *p*H of the test liquor was recorded from the results of examination by B.D.H. comparator. The indicators used were Chloro-phenol Red and Bromo-cresol Green of original B.D.H. make. The results were recorded every 24 hours The wort was roused and the 'heads' were removed after the first two days of fermentation.

Experimental Results

Type I (M_5)—This strain of *S. cerevisiae* was known to be a typical beer-fermenting yeast, imported from the United Kingdom. The growth and multiplication of the cells were neither too slow nor too fast. From Table 1, it would appear that the rate of consumption of sugar during the first 24 hours was rather faster than in the later periods of growth, when it became steady, and the process continued until the sixth day of fermentation, after which there was no further reduction of the sugar-content of the fermenting wort. Reduction of sugar, as will as fall in gravity and total solids took place, with the only exception that during the first 24 hours no rapid change of specific gravity or the reduction in the quantity of total solid was marked. The *p*H showed a very

slow accumulation of organic acids in a strong biologically buffered wort. On the fourth day of fermentation the rate of formation of acids increased a little, and then no further change took place. At the end of the sixth day all the processes, e.g., consumption of sugar, change of *p*H, reduction in specific gravity and quantity of total solids stopped indicating that the primary fermentation was completed.

Table 1. *Effect of the growth of pitching yeast in beerwort during first seven days*

Pitching yeast	Days after pitching	Sp. Gr.	Total Solid (°Brix)	Sugar (Maltose%)	*p*H
	0	1.040	10.0	7.5	5.6
	1	1.039	9.9	5.2	5.5
	2	1.035	8.9	4.8	5.3
M_5	3	1.030	7.8	4.2	5.2
	4	1.022	5.6	3.8	5.1
	5	1.016	4.2	2.7	4.7
	6	1.010	2.8	1.3	4.6
	7	1.010	2.8	1.3	4.6
	0	1.040	10.5	7.5	5.6
	1	1.038	9.7	5.9	4.8
	2	1.022	7.1	4.1	4.5
B_1	3	1.013	4.7	2.4	4.3
	4	1.011	3.7	1.7	4.2
	5	1.010	3.5	1.4	4.0
	6	1.010	3.5	1.4	4.0
	7	1.010	3.5	1.4	4.0
	0	1.040	10.5	7.5	5.6
	1	1.036	9.6	5.3	5.2
	2	1.014	3.8	2.7	4.1
B_2	3	1.010	3.5	1.3	4.0
	4	1.010	3.5	1.3	4.0
	5	1.010	3.5	1.3	4.0
	6	1.010	3.5	1.3	4.0
	7	1.010	3.5	1.3	4.0
	0	1.040	10.5	7.5	5.6
	1	1.038	9.7	5.2	5.2
	2	1.036	9.2	4.8	5.0
B_3	3	1.022	5.8	3.6	4.5
	4	1.020	5.2	3.3	4.4
	5	1.018	4.8	2.7	4.4
	6	1.010	2.8	1.6	4.2
	7	1.010	2.8	1.6	4.2
	0	1.040	10.5	7.5	5.6
	1	1.040	10.5	7.5	5.6
	2	1.039	10.2	6.9	4.8
B_{10}	3	1.030	7.8	5.7	4.4
	4	1.012	4.5	2.2	4.2
	5	1.010	3.4	1.4	4.0
	6	1.010	3.4	1.4	4.0
	7	1.010	3.4	1.4	4.0
	0	1.040	10.5	7.5	5.6
	1	1.036	9.4	6.1	5.5
	2	1.034	8.8	6.0	5.3
B_3+B_{10}	3	1.028	7.4	5.8	5.1
	4	1.022	5.8	4.2	5.0
	5	1.016	4.2	3.1	4.6
	6	1.009	2.4	1.4	4.3
	7	1.009	2.4	1.4	4.3

Types II and III (B_1 *and* B_2)—These two strains of *S. cerevisiae* were obtained from a distillery, where these were used for their capability of starting vigorous fermentation in the early stage of multiplication. The sugar consumption was not too fast during the first 24 hours. The reaction became extremely vigorous on the third day of fermentation. The fall in the amount of total solid and the reduction of specific gravity followed the same course with the consumption of sugar. The increase in the acidity took place suddenly during the second day of fermentation in B_2, and no further change was observed until the end of fermentation. The course of reaction in B_1 was somewhat different from that of B_2. It could, however, be concluded from the experimental results presented here that these two strains possessed higher attenuating power.

Type IV (B_3)—This was a purified culture and a selected strain of *S. cerevisiae* obtained from an imported Italian wine. It appears from the experimental results that the two types M_5 and B_3, although obtained from two distinctly different sources, had nearly idential fermenting behaviour. In the case of the strain B_3 also the wort was left with a very small quantity of residual fermentable sugar at the end of the sixth day.

Type V (B_{10})—This strain of yeast was obtained from a laboratory stock and purified afterwords in beerwort-agar, and was used for fermentation. The special feature of this strain was that during the first two days of incubation there was very little change in the compostion of the wort. It would appear from the table that the multiplication of yeast started after two days of fermentation, but the reaction increased suddenly. On the fourth day the process slowed down and totally stopped in course of another 24 hours. The rise in the acidity of the wort during the first 48 hour could be explained as due to the prolonged lag phase of the yeast during this period and the consequent increase in the lactic acid fermentation at the early stage.

Type VI (B_3+B_{10})—It has been reported that if a mixture of pure cultures of two different strains of yeast is used for pitching beerwort, the behaviour of the mixed strain would show an intermediate characteristic during fermentation. In the present work the strains B_3 and B_{10} were two extreme types as regards their capability of fermenting beerwort. With an idea of obtaining blended characters in the mixed strain dilutions of cells were made with sterile water and taken in such proportions that the mixture would contain approximately equal number of cells from each of these strains. The rate of attenuation of beerwort, however, gradually acquired the characteristics of the slow fermenting type B_3.

Discussion

The strains of yeast used were of low-flocculating type and the experiments showed that the period of complete attenuation (the grade required for beer) was not more than seven days, which was taken to be suitable for beer fermentation.

Of all the yeast strains used for the experiments, M_5 and B_3 attenuated the beerwort at a uniform rate, and reactions stop at the end of the sixth day from the date of pitching. The most striking results were obtained with the strains B_1 and B_2. These cultures possessed high attenuating power, and the reactions stopped in both these cases almost after the third day of fermentation. The strain B_{10}, however, showed a distinctly different nature of reaction. It remained almost inactive during the first two days of fermentation (excepting the production of acids). The fermentation started at the end of the second day, and the rate went on increasing suddenly until it stopped almost at the end of the fourth day. The mixed type B_3+B_{10} was used in this experiment with a view to study its behaviour towards the beerwort. Although it was expected that the resulting reaction would be a different one, yet the results showed that the characters of the slow-fermenting strain B_3 dominated.

There was a considerable controversy amongst the earlier workers about the superiority of a pure culture of yeast over a mixture of selected strains in the production of flavour, brilliancy and general qualities of the finished beer. The use of pure yeasts had not been adopted in several countries for top-fermentation beer. Van Laer (1894) recommended that a mixture of selected races of yeast is almost essential for a successful production of finished beer. This procedure was, however, contradicted by Jörgensen (1894), who argued that in order to introduce an element of certainty into fermenting conditions it was indispensable to use a single race of pure yeast. By a continuous trial Miller and Hyde (1894) confirmed the results obtained by Jörgensen. Brown (1934) suggested a modified system of using two or more selected races of yeast together, and his experiments proved a success after extensive trials.

It was observed in the present investigation, that single strains of pure yeast (excepting B_1 and B_2, which are found to be high alcohol-producing yeasts, and therefore, unsuitable for beer fermentation) were suitable for fermenting beerwort. After giving them a few trials, it was possible to forecast the nature of attenuation of the wort and the quality of the finished beer produced by them. The pure strain B_{10}, although attenuated the wort to a desirable extent, failed to produce the typical flavour. The desired flavour of the finished beer could only be obtained from brews fermented by the strains M_5 and B_3 . It is interesting to note that the flavour developed in the beer by the mixed strain B_3 and B_{10} did not correspond to that of either B_3 or B_{10} alone. The flavour was quite different and was not at all suitable for finished beer. From the results stated above,it could be concluded that a tried single strain of pure yeast could be safely used for fermenting beerwort, while the use of mixed strains for this purpose should be considered rather risky and with uncertain effects, as the strain getting the upper hand and guiding the course of fermentation cannot be predicted without extensive trials.

Acknowledgement

We wish to express our sincere thanks to Dr. P. N. Bhaduri, Professor of Botany, Presidency College, Calcutta, for his continued interest and encouragement in the work which was carried out in the Research Laboratory of the Bengal Breweries Ltd., Calcutta. during 1942 and 1944.

(*Received for publication 8 April 1957*)

References

Brown. B. M. (1934). Purification of pitching yeast. *Jour. Inst. Brew.*, **40**, 9.

Jörgensen, A. (1894). Pure yeast for top fermentation of beers. *Ibid*, **7**, 27.

Miller, A. K. and Hyde, C. F. (1894). Secondary fermentation of beers. *Trans. N. Eng. Brew.*, **3**, 39.

Van Laer, H. (1894). Selected races of yeast for top fermentation. *Journ. Inst. Brew.*, **7**, 55.

Ind. J. mycol. Res., **2** (1 & 2), 57—64 (1956).

THELEPHORACEAE OF THE MUSSOORIE HILLS—I

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(With Plates I & II and 2 Text-figures)

Eight species of Thelephoraceae collected from the Mussoorie Hills have been described. Of these, four are new records from India while *Craterellus odoratus* Schw. ex Fr. var. *solidostipite* is a new variety.

Introduction

An excursion is made every year from the Botany Department of the Panjab University to the Mussoorie Hills in the North-Western Himalayas (2,000–7,000 ft.) to study the cryptogamic flora. The Thelephoraceae form a part of the collection by the authors. This contribution describes seven known species and one new variety.* Of six dozens species of Thelephoraceae reported from India, about three dozens were described by Banerjee (1935) and Bagchee and Bakshi (1954). The rest are only isolated reports by different workers at different times (Butler & Bisby, 1931; Mundkur, 1938).

Descriptions of Species

1. *Craterellus odoratus* Schw. ex Fr. var. *solidostipite* var. nov.

(Plate I, fig. 1; text-fig. 1, a—c)

Usque 16 *cm. alta: pileus multiramosa: stipite solido: hymenio plano: ad terram.*

Mossy Falls Estate, Mussoorie, India: September 14, 1954; Herb no. 181.

Fructifications annual; fleshy, brittle; stipitate; usually gregarious, sometimes solitary, or in caespitose clusters of 2–3 fruit-bodies; 6–16 cm. high. *Pileus* more or less infundibuliform, profusely and deeply lobed, lobes small to large, irregular and oriented variously and overlapping each other; orange yellow; 5–10 cm. in diameter. *Context* light orange yellow. *Margin* entire, concolorous, recurved. *Hymenial surface* lighter coloured than the upper surface of the pileus, yellow-orange; mostly smooth, sometimes irregularly rugulose or clearly lamelliform, or daedaloid at places, lamellae mostly towards the periphery but absent near the margin, very thin, radial and mostly wavy. *Hymenium* decurrent. *Stipe* central; yellow-orange; solid; uniformly cylindrical but sometimes grooved, inflated above towards the pileus, mostly straight, sometimes slightly

* The materials have been kept in the Herbarium of the Panjab University. Duplicate materials in alcohol-formalin have been deposited in the Herbarium, Royal Botanic Gardens, Kew, England.

bent ; 2–9 cm. long and 0.5–1.5 cm. broad. *Hyphae* thin-walled, branched, septate, septa mostly at short intervals, clamp-connexions present almost at every septum, sub-hyaline, stainable, 3–8μ wide, hyphal cells 20–100μ long. *Basidia* clavate or elongated, hyaline. 8–12.5$\times$4–6μ ; sterigmata 4, mostly incurved, 4–6μ long. *Basidiospores* hyaline to sub-hyaline, oblong to ellipsoid or obovoid, papillate, papilla minute, smooth, thin-walled, mostly uniguttulate, 4–8$\times$4–6μ.

Growing on soil near Mossy Falls under Oak (*Quercus incana* Roxb.) forest; Mussoorie; September 14, 1954; Herb. no. 181.

This fungus was found growing luxuriantly on soil in the Mossy Falls Estate. It is easily recognized by its mushroom like habit, large size, much branched and lobed, orange-yellow pileus, firm and solid stipe, and broadly ellipsoid, smooth basidiospores (4–8$\times$4–6μ). It belongs to *Craterellus odoratus* Schw. ex Fr. except in that its stem is solid and firm. In the latter respect it resembles *C. cantharellus* Schw. ex Fr. but differs from the latter in its branched pileus and mostly even hymenium. Thus, this fungus is clearly intermediate between *C. odoratus* and *C. cantharellus* and is considered here as a new variety of *C. odoratus*. The varietal name *solidostipite* is proposed here to designate its stem.

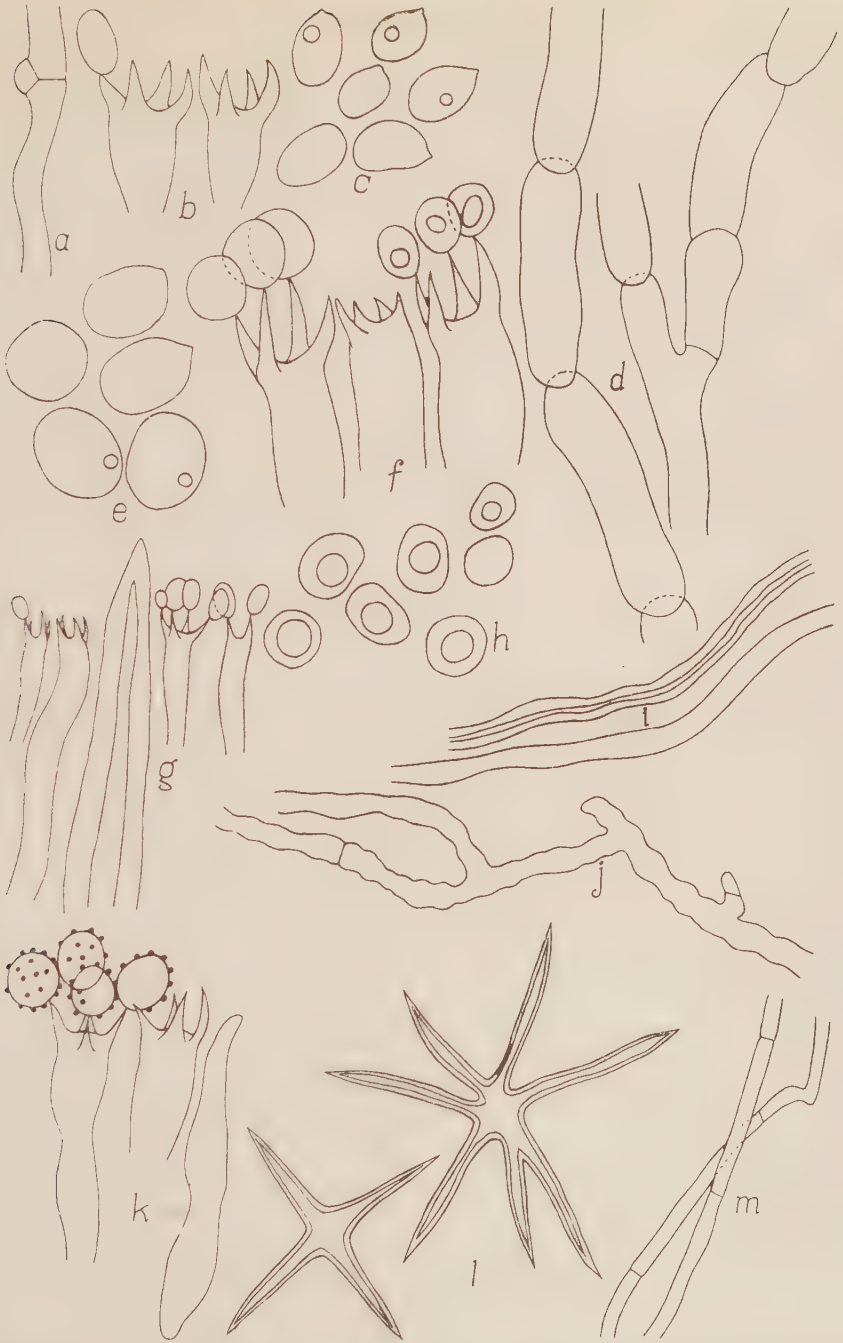
2. *Craterellus sinuosus* Fr., Epicr., 533, 1836–1838.

(Plate I, figs. 2 and 5; text-fig. 1, d, f, e)

Fructifications annual; fleshy; stipitate; gregarious to scattered, sometimes caespitose; 1.5–7 cm. high. *Pileus* infundibuliform, when young orbicular and depressed at the centre or tubiform; upper surface smooth to rough or floccose, light brown to dark brown or blackish brown; up to 4.5 cm. in diameter. *Margin* wavy to dentate, outcurved, concolorous to light coloured. *Hymenial surface* whitish grey or ash coloured; mostly smooth, later becoming irregularly wrinkled, sometimes slightly lamellate. *Hymenium* somewhat decurrent. *Stipe* simple; central; free, sometimes confluent ; light brown to dark brown ; cylindrical, sometimes flattened and grooved, expanded above into the pileus; solid, sometimes hollow; 1–5.5 cm. long and 2–8 mm. thick. *Hyphae* hyaline to sub-hyaline, thin-walled, branched, inflated, closely septate, without clamp-connexions, 4–10μ wide; hyphal cells up to 40μ long. *Basidia* clavate to elongate, sub-hyaline to light brown, 5–8μ broad; sterigmata 4, incurved, mostly 4–7 (10)μ long. *Basidiospores* sub-hyaline, ovoid to broadly ellipsoid, minutely papillate, smooth, uniguttulate to vaguely guttulate, 8–10$\times$5–8μ.

Growing on soil; The Park Toll Bar, Mussoorie; September 6, 1954; Herb. no. 182. The fungus is common in dark, shady, humid places along recently eroded hill slopes and growing out of holes or depressions; Chakrata Toll, Mussoorie; September 2, 1953; Herb. no. 183. On soil under Oak forest; Jabber Khet, Mussoorie; September 4, 1953; Herb. no. 184. On soil under Oak forest (*Quercus incana* Roxb.); Chakrata Toll, Mussoorie; September 15, 1954; Herb. no. 185 ; a new record from India.

The fungus is easily recognized by its infundibuliform pileus, whitish grey or ash coloured hymenium, and smooth, broadly ellipsoid basidiospores (8–10$\times$5–8μ).



Text fig. 1. *a—c*, *C. odoratus* var. *solidostipite* : a, Hypha with clamp-connexion, × 750; b, Basidia and c, Basidiospores, × 1150. *d—f*, *C. sinosus* : d, Hyphæ with inflated cells, × 750; e, Basidiospores and f, Basidia, × 1150. *g—j*, *H. semistupposa* : g, Basidia and setae, × 1150; h, Basidiospores, × 1150; i, Skeletal and j, Generative hyphae, × 750 *k—m*, *A. cervicolor* : k, Basidia, Basidiospores and Gleocystidium, × 1150; l, stellate Setae, × 480; m, thin-walled Hyphae, × 750.

3. *Hymenochaete semistupposa* Petch, Ann. R. bot. Gdns. Peradeniya **9**, 278, 1925.

(Plate I, fig. 3; text-fig. 1, g–j)

Fructifications annual; coriaceous, becoming hard and brittle on drying; entirely resupinate and abundantly cracked, cracks anastomosing, extending up to 1 foot. *Hymenial surface* dark brown to almost black; smooth. *Margin* wavy, entire, thin, lighter coloured than the hymenium. *Subiculum* 600–750μ thick, dark brown in colour. *Hyphae:* skelatal hyphae highly thick-walled, lumen completely obliterated in most cases, brown, unbranched, aseptate or with pseudosepta, clamp-connexions absent; 2.5–4μ wide; generative hyphae thin-walled, light brown, branched, septate, without clamp-connexions, 2–4μ wide. *Hyphal pegs* hyaline, mostly pyramidal, often surround the projected parts of setae, projecting about 10–20μ beyond the hymenium. *Setae* very abundant, light brown to dark brown, subulate, mostly embedded in the subiculum, some projecting out and extending up to 40μ beyond the hymenial layer, dimension 30–120 × 6–10μ. *Basidia* clavate or elongated; Sterigmata 4, sometimes 2, straight, 3–6μ long. *Basidiospores* light brown, sub-globose or ellipsoid, smooth uniguttulate, thin-walled, 4–8 × 3–5μ.

Growing on stumps and exposed roots of *Quercus incana* Roxb.; Dhobi Ghat, Mussoorie; September 6, 1953; Herb. no. 186; a new record from India.

This species is characterized by the resupinate, dark brown to almost black fructifications which become abundantly cracked, mostly embedded setae, and light brown, smooth, subglobose to broadly ellipsoid basidiospores (4–8 × 3–5μ).

4. *Asterostroma cervicolor* (Berk. and Curt.) Massee, J. Linn. Soc. Bot. **25**, 155, 1889.

(Plate I, fig. 4; text-fig. 1, k–m)

Fructifications annual; entirely resupinate; brittle; resupinate patches extending up to 1.5 ft., encroaching upon mosses etc. that come in its way. *Margin* irregular, yellowish pink, fluffy, sterile. *Hymenial surface* smooth; ochraceous pink, becoming cream coloured or brownish on drying. *Subiculum* 60–80μ thick. *Hyphae* thin-walled, branched, closely septate, sometimes swollen near the septa, hyaline, slightly stainable, without clamp-connexions, 1.5–3.5μ wide; abundant crystals forming a regular compact layer in the subiculum present. *Stellate setae* abundant in the sub-hymenium as well as lower down in the subiculum but more abundant above the layer of crystals, dark brown, not stainable, with 3–6 radiating arms, thick-walled, dimension of the arms 20–50 × 2.5–4μ. *Gloeocystidia* abundant, mostly arising from the sub-hymenium, some extending up to the hymenial surface, broader below but narrowed above, hyaline to sub-hyaline, 20–52 × 4–14μ. *Basidia* clavate to elongate, hyaline, 10–14 × 4–6μ; sterigmata 4, straight, sometimes curved. *Basidiospores* sub-hyaline to faintly brown, globose, with numerous tuberlces, 3–4.5μ in diameter.

Growing on stumps and decaying logs of wood; Hardwar Road, Dehra Dun; August 16, 1954; Herb. no. 187; a new record from India.

Text fig. 2. *a—d, S. elegans* : a, Skeletal and b, Generative hyphae, × 750; c, Basidia, and a Gleocystidium, × 1150; d, Basidiospores, × 1150. *e—h, S. purpureum* : e, Skeletal, and f, Generative hyphae, and also a hypha with differentiating Vesicular bodies, × 750 g, Basidia, Gleocystidium and h, Basidiospores, × 1150. *i—k, S. gausapatum* : i, Thin-walled or slightly thick-walled hypha, × 75c; j, Basidia and a Conducting organ, × 1150; k, Basidiospores, × 1150. *l—n, S. hirsutum* : l, Thick-walled to almost solid hyphae, × 750; m, Basidia and a Conducting organ, × 1150; n, Basidiospores, × 1150.

This species is characterized by the large, resupinate, ochraceous pink fructifications with irregular, sterile margin, abundant stellate setae and gloeocystidia, and globose, prominently tubercled basidiospores (3–4.5μ). In the Mussoorie collection gloeocystidia are all embedded and do not project out of the hymenial layer while they are reported to project up to 25μ beyond the hymenial layer (Burt, 1924).

5. *Stereum elegans* (Meyer ex Fr.) Fr. sensu Petch, Ann. R. bot. Gdns., Peradeniya **9**, 259–298, 1925.

(Plate II, fig. 6; text-fig. 2, a–d)

Fructifications annual; thin, tough, sub-cartilagious, becoming slightly brittle on drying; erect; stipitate; in fasciculate clusters; up to 8 cm. high. *Pileus* flabelliform or infundibuliform; confluent with one another; deeply cleft on one side, or lobed, lobes large to small or irregular; upper surface irregularly and radially ridged, concentrically zoned, deep cream coloured, on drying, turning greyish brown with darker tips and shrivelling up; 1.5–5.5 cm. broad, 1.2–4 cm. high and up to 1 mm. thick. *Margin* slightly to deeply cleft, thin, lighter coloured. *Hymenial surface* cream coloured, lighter coloured than the upper surface, rough, ridged more or less radially, concentrically zoned. *Stipe* lateral or excentric, mostly confluent, solid, cylindrical to flattened, expanding above into the pileus, rough, dark brown, 1.4–3.5 cm. long and 2–3 mm. wide. *Hyphae*: skeletal hyphae thick-walled, mostly simple, rarely branched, flexuous, without clamp-connexions, hyaline, non-strainable, 3–5μ wide, wall 1–1.5μ thick ; generative hyphae thin-walled, sparsely branched, distantly septate, with abundant large clamp-connexions, nodose-septate. *Gloeocystidia* abundant, arising from the sub-hymenium, broader below, but narrowed above, sub-hyaline, deeply stainable, 7–9μ wide near the top and 60–95μ long. *Basidia* clavate or elongated, hyaline, about 4–5μ broad ; sterigmata 4, sometimes 2, straight, 3.2–4μ long. *Basidiospores* hyaline, broadly ellipsoid or obovate, sometimes sub-globose, smooth, thin-walled, uniguttulate, 3.5–5.5$\times$ 3–4μ.

Growing on dead roots and stumps of *Quercus incana* Roxb.: Saharanpur Road, Dehra Dun; July 31, 1953; Herb. no. 188.

This species is easily recognized by its densely fasciculate, deep cream coloured fructifications deeply cleft on one side and with a lighter coloured margin, abundant gloeocystidia, and hyaline, smooth, ovoid to sub-globose basidiospores (3.5–5.5 $\times$ 3–4μ). The Dehra Dun collection closely resembles *S. elegans* Fries reported from Bengal by Banerjee (1935) except that the Dehra Dun specimens are larger and possess abundant gloeocystidia and clamp-connexions in the generative hyphae.

6. *Stereum purpureum* Pers., Roemer Neues Mag. Bot **1**, 10, 1794.

(Plate II, fig. 7; text-fig. 2, e–h)

Fructifications annual; cartilagenous; mostly effuso-reflexed; imbricate. *Pileus* light brown to dark brown with upper surface concentrically zoned and hairy or villose-tomentose, irregularly lobed, narrowed below into

a stem-like base, 2–4.8 cm. wide, up to 300μ thick; hairs in fascicles, sub-hyaline, thick-walled, lumen completely obliterated, unbranched. *Margin* closely incised, slightly incurved, thin, lighter coloured. *Hymenial surface* lighter coloured than the upper surface, light brown, smooth or slightly wrinkled radially. *Hyphae:* skeletal hyphae unbranched, aseptate, highly thick-walled, lumen completely obliterated in most cases, without clamp-connexions, stainable, 2–4μ wide; generative hyphae thin-walled, branched, septate, with clamp-connexions, stainable, 2-4μ wide; *Vesicular bodies* quite conspicuous, embedded in the sub-hymenium, globose to pyriform, thin-walled, deeply stainable, 10–20 × 6–10μ. *Gloeocystida* sparsely present, arising from the sub-hymenial layer and extending up to the hymenial layer, sub-hyaline, deeply stainable, broader below and narrowed above, 13–30μ long and 4–8μ wide. *Basidia* clavate, sub-hyaline, 6–8 × 2–3μ ; sterigmata 4, sometimes 2, straight, 2-4μ long. *Basidiospores* hyaline, narrowly ellipsoid, papillate, smooth, uniguttulate, 4–5 × 2–3μ.

Growing on stumps of trees; The Park Road, Mussoorie; August 26, 1954; Herb. no. 189.

This species is characterized by its brown, tomentose and mostly effuso-reflexed fructifications, prominent vasicular bodies embedded in the sub-hymenium and narrowly ellipsoid, smooth basidiospores (4–5 × 2–3μ). Gloeocystidia have been noted *sparsely developed* in the Mussoorie collection.

7. *Stereum gausapatum* Fr., Hym. Eur., 638, 1874.

(Plate II, fig. 8; text-fig. 2, i–k)

Fructifications annual; tough, coriaceous; effuso-reflexed; imbricate; resupinate portion up to 37 cm. wide. *Pileus* with brown upper surface, paler at the periphery; concentrically zoned, zones faintly reddish brown in colour; hairy; lobed; up to 2 cm. high and 645μ in thickness ; hairs shaggy, brown, thick-walled, rigid, erect, radially arranged. *Margin* dentate, thin, lighter coloured. *Hymenial surface* brown to dark brown, paler at the periphery, smooth or uneven due to irregular radial wrinkles. *Hyphae* thin-walled to slightly thick-walled, hyaline, branched, distantly septate, stainable, clamp-connexions absent, 1.5–4μ wide. *Conducting organs* abundant, with reddish brown contents, broader below but narrowed above, 40–70 × 6–12μ. *Basidia* clavate, hyaline, 8–12 × 2.4–4μ; sterigmata 4, sometimes 2, straight or slightly incurved, 4–6μ long. *Basidiospores* hyaline, narrowly ellipsoid, papillate, papilla minute, smooth, 4–7 × 2–4μ.

Growing on logs and stumps of Oak (*Quercus incana* Roxb.) ; The Park, Mussoorie; August 23, 1954; Herb. no. 190; a new record from India.

S. gausapatum is recognized by its brown, effuso-reflexed fruit-body, imbricate growth, villose to shaggy hairs, darker hymenium which bleeds when cut or bruised, abundant conducting organs, and smooth, narrowly ellipsoid basidiospores (4–7 × 2–4μ).

8. *Stereum hirsutum* Willd. ex Fr., Epicr., 549, 1838.

(Plate II, fig. 9; text-fig. 2, l–n)

Fructifications perennial; leathery; effuso-reflexed, extending up to 20 cm.; imbricate; up to 5 cm. high. *Pileus* with upper surface yellowish brown, concentrically zoned, irregularly lobed, surface profusely hairy,

7×5 cm. wide and up to 540μ thick (excluding the tomentose layer); hairs yellowish brown, becoming deeper coloured in old specimens, highly thick-walled so as to obliterate the lumen completely. *Margin* entire, wavy, lighter coloured, involuted when dry. *Hymenial surface* orange coloured, smooth. *Hyphae* thick-walled, unbranched, aseptate, sub-hyaline, slightly stainable, clamp-connexions absent, $1.4–6\mu$ wide, wall $1.4–2.4\mu$ thick. *Conducting organs* filled with orange contents, elongated, sinuous, usually broader below but narrowed above, tips rounded or prolonged into a knob-like portion, slightly stainable, $160–720 \times 3.2–5.6\mu$. *Basidia* elongated, sub-hyaline, $4–6\mu$ broad; sterigmata 4, sometimes 2, straight, $2.4–4.8\mu$ long. *Basidiospores* hyaline, narrowly ellipsoid, smooth, aguttulate; $4–6 \times 1.8–2.5\mu$.

Growing on stumps of trees; Burning Ghat, Mussoorie; August 23, 1954: Herb. no. 191.

This species is very common in the Mussoorie Hills and is easily recognized by its yellowish brown, strigose-hirsute, effuso-reflexed pileus, and narrowly ellipsoid basidiospores ($4–6 \times 1.8–2.5\mu$). This Mussoorie collection shows abundant conducting organs filled with orange contents. No conducting organs were reported for the species by Burt (1920). However, conducting organs have been reported for the species by Banerjee (1935) and rarely observed by Bagchee and Bakshi (1954).

Acknowledgement

The authors are deeply indebted to Dr. D. A. Reid, the Royal Botanic Gardens, Kew, for help in the identification of the species and to Prof. P. N. Mehra for conducting the excursion and for providing facilities and encouragement.

References

Bagchee, K. and Bakshi, B. K. (1954). Studies on Indian Thelephoraceae—I. Some species of *Stereum*, *Peniophora* and *Corticium*. *Indian For. Bull.* No. 166, N. S. (Mycology), 1–2.

Banerjee, S. N. (1935). Thelephoraceae of Bengal—I. *J. Ind. Bot. Soc.*, **14** : 13–48.

Burt, E. A. (1920). The Thelephoraceae of North America–XII. *Ann. Mo. bot. Gdn.*, **7** : 81–248.

Burt, E. A. (1924). The Thelephoraceae of North America. XIII. *Ann. Mo. bot. Gdn.*, **11** : 1–36.

Butler, E. J. and Bisby, G. R. (1931). *The fungi of India*, Sci. Mono. 1, Indian Coun. Agric. Res.

Mundkur, B. B. (1938). *Fungi of India*, Suppl. I. Sci. Mono. 12, Indian Coun. Agric. Res.

EXPLANATION OF PLATES

Plate I

Fig. 1. *Craterellus odoratus* Schw. ex Fr. var. *solidostipite* var. nov.
Figs. 2 & 5. *Craterellus sinnosus* Fr.
Fig. 3. *Asterostroma cervicolor* (Berk. and Curt.) Massee.
Fig. 4. *Hymenochaete semistupposa* Petch.

Plate II

Fig. 6. *Stereum elegans* (Meyer ex Fr.) Fr.
Fig. 7. *S. purpureum* Pers.
Fig. 8. *S. gausapatum* Fr.
Fig. 9. *S. hirsutum* Willd. ex Fr.

(*Received for Publication 30 March 1957*)

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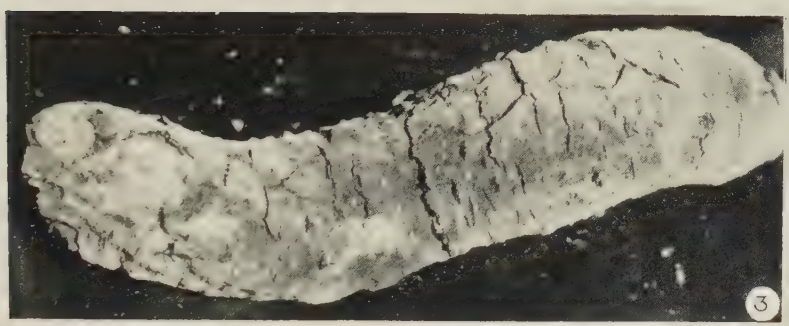
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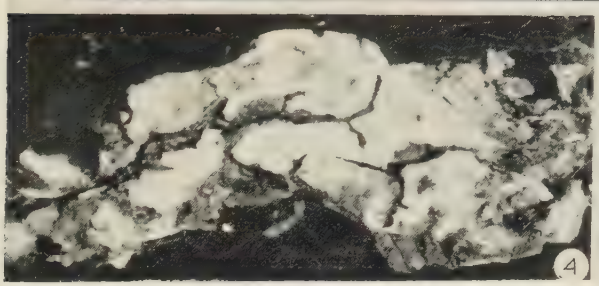
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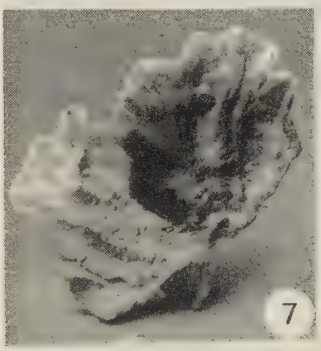
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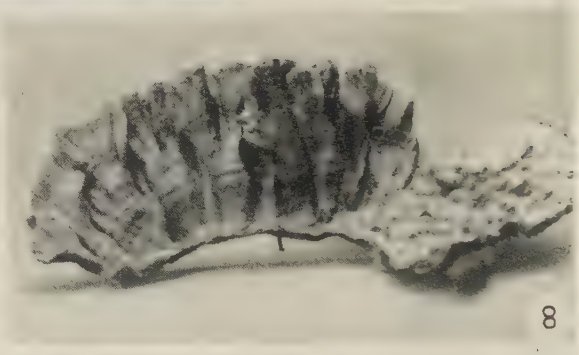
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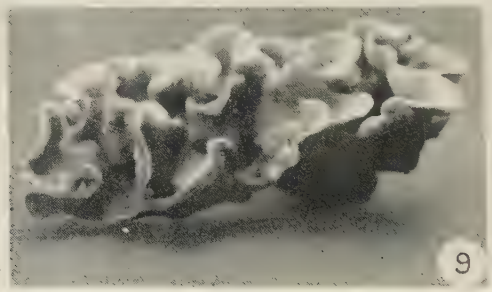
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Ind. J. mycol. Res., **2** (1 & 2), 65–70 (1956).

FUNGI OF AJMER (RAJASTHAN)—I.

By N. C. JOSHI

Botany Department, Government College, Ajmer

Fifty-five species of both pathogenic and non-pathogenic fungi belonging to different groups and their respective hosts have been recorded for the first time from Ajmer and are listed in this paper.

Since 1952, the writer with the co-operation of his colleagues and post-graduate students started collecting fungi from Ajmer in order to have some idea about their occurrence and distribution in this locality. These collections have been kept in the Mycology Herbarium of the Botany Department, Government College, Ajmer and some interesting ones have been deposited at C.M. I, Kew, Surrey, England and Herb. Crypt. Indiae, Orient, New Delhi, India.

List of Fungi

1. Bremia lactucae Regl. (*Sacc**. VII : 244; *B. & B***., 2).

On living leaves of *Sonchus oleraceous* L. (Compositae) Govt. College Campus, 6-8-1950, Leg A. N. P., Myco. Herb. No. 1 (Conidial stage).

2. Albugo candida (Pers.) Léveille (*Sacc.* VII : 334; *B. & B.*, 3)

On living leaves of *Brassica campestris* L. (Cruciferæ), Bot. Garden, Govt. College, 15-8-1950, Leg N. C. J., Myco. Herb. No. 2 (Conidial stage only); of *Raphnus sativus* L. (Cruciferæ), Victoria Hospital, 3-11-1952, Leg N. C. J., Myco. Herb. No. 6 (Conidial Stage).

3. Albugo ipomoeae-aqualigae Sawada

On living leaves of *Ipomoea reniformis* Chois (Convulvolaceae), Taragarh, 8-8-1952, Leg N. C. J., Myco. Herb. No. 3 (Conidial stage).

4. Albugo portulacae (D.C.) Léveille (*Sacc.* VII : 235; *B. & B.*, 3)

On living leaves of *Portulaca oleracea* L. (Portulacaceae), Government College Campus, 8-8-1952, Leg. N. C. J., Myco. Herb. No. 4 (Conidial stage).

5. Albugo bliti (Biv.) deBary (*Sacc.* VII : 236; *B. & B.*, 2)

On living leaves of *Amarantus paniculatus* L. (Amarantaceae), Bot. Garden, Govt. College, 15-8-1952, Leg N. C. J., Myco. Herb. No. 5

* *Sacc.* = Saccardo, P.A.

** *B. & B.* = Butler, E. J. & Bisby, G. R.

(Conidial stage); of *Digera arvensis* Forsk. (Amarantaceae), Pushkar valley 10-8-1952, Leg N. C. J., Myco. Herb. No. 8 (Conidial stage).

6. Albugo platensis Speg. (*Sacc.* XI : 242; *B. & B.*, 3).

On living leaves of *Boerhaavia diffusa* L. (Nyctaginaceae), Civil lines, 6-9-1953, Leg N. C. J., Myco. Herb. No. 6 (Conidial stage).

7. Perenospora effusa (Grev.) Raben. (*Sacc.* VII : 256; *B. & B.*, 4)

On living leaves of *Chenopodium album* L. (Chenopodiaceae), Tabeji Farm, 2-2-1953, Leg N. C. J., Myco. Herb. No. 9 (Conidial stage).

8. Perenospora trifolium deBary (*Sacc.* VII : 249; *B. & B.*, 4)

On living leaves of *Melilotus alba* Desv. (Leguminosae), Pushkar Fields, 6-10-1954, Leg N. C. J., Myco. Herb. No. 10 (Conidial stage).

9. Perenospora parasitica (Pers.) deBary (*Sacc.* VII : 249; *B. & B.*, 4)

On living leaves of *Brassica campestris* L. (Cruciferae), Link Road, 6-10-1953, Leg N. C. J., Myco. Herb. No. 11 (Conidial stage).

10. Perenospora celsiae Syd. (*Sacc.* XXIV : 60; *B. & B.*, 4)

On living leaves of *Celsia coromandeliana* Vahl. (Scrophulariaceae), Foysagar, 2-2-1953, Leg N. C. J., Myco. Herb. No. 12 (Conidial stage).

11. Phytophthora colocasiae Raciborski (*Sacc.* XVI : 292; *B. & B.*, 5)

On living leaves of *Colocasia antiquorum* Schott. (Araceae), Beer Village, 2-3-1953, Leg N. C. J., Myco. Herb. No. 13 (Conidial stage).

12. Sclerospora gramnicola (*Sacc.*) Schroeter (*Sacc.* VII : 238 : *B. & B.*, 7)

On living leaves and the inflorescence of *Pennisetum typhoideum* Rich. (Gramineae), Adarsh Nagar, 11-11-1952. Leg N. C. J., Myco. Herb. No. 14 (Conidial and oosporic stages).

13. Rhizopus nigricans Ehrenberg (*Sacc.* VII : 212; *B. & B.*, 9)

Isolated from the soil, Govt. College Campus, 6-9-1953, Leg N. C. J., Myco. Herb. No. 15 (Asexual stage).

14. Aspergillus candidus Link. (*Sacc.* IV : 66; *B. & B.*, 139)

A contaminant in the culture media in the Botany Laboratory, 3-9-54, Leg N. C. J., Myco. Herb. No. 16 (Conidial stage).

15. Capnodium ramosum Cooke (*Sacc.* XI : 271; *B. & B.*, 18)

On living leaves of *Mangifera indica* L. (Anacardiaceae), Happy valley, 3-9-1954, Leg N. C. J., Myco. Herb. No. 17 (Conidial and the perithecial stages).

16. Capnodium citri Berk. and Desen. (*Sacc.* I : 78)

On living leaves of *Citrus* Sp. (Myrtaceae), Pushkar, 3-9-1953, Leg B. S., Myco. Herb. No. 18 (Conidial and perithecial stages).

17. Erysiphe polygoni D.C. (*Gaur*, 1949; *Joshi*, 1956)

On living leaves and twigs of *Cuminum cyminum* L. (Umbelliferae), Kekri, 3-3-1953, Leg N. C. J., Myco. Herb. No. 19 (Conidial stage).

18. Erysiphe cichoracearum D.C. (*B. & B.*, 22)

On living leaves and twigs of *Coccinia indica* (Cucurbitaceae), Nasirabad Road, 2-2-1953, Leg A. N. P., Myco. Herb. No. 20 (Conidial and perithecial stages).

19. Protomyces macrosporus Unger. (*Sacc.* VII : 319 ; *B. & B.*, 9)

On living leaves, stem and branches of *Coriandrum sativum* L. (Umbelliferae), Civil Lines, 1-1-1950, Leg B. D. T., Myco. Herb. No. 21A (Resting spores).

20. Protomycopsis ajmerensis Gupta (*Gupta*, 1956)

On living leaves of *Sesbania aculeata* Pers. (Leguminosae), Anasagar (Lake), 8-9-1950, Leg B. B. T., Myco. Herb. No. 21B (Resting spores).

21. Phyllactinia corylea var. subspiralis Salmon (*B. & B.*, 35)

On living leaves of *Dalbergia sissoo* Roxb. (Leguminosae), Kishangarh Road, 2-2-1952, Leg N. C. J., Myco. Herb. No. 22 (Conidial and perithecial stages).

22. Phyllachora cynodontis (Sacc.) Niessel (*Sacc.* II : 602 ; *B. & B.*, 33)

On living leaves of *Cynodon dactylon* Pers. (Gramineae), Civil lines, 1-1-1953, Leg N. C. J., Myco. Herb. No. 23 (Perithecial stage).

23. Xylaria aspersa Massee (*Sacc.* XVI : 444; *B. & B.*, 41)

On soil, Bot. Dept. Campus, 2-3-1952, Leg N. C. J., Myco. Herb. No. 24.

24. Psalliota campestris L. (*Sacc.* V : 997; *B. & B.*, 84)

On soil, Foysagar, 8-9-1953, Leg N. C. J., Myco. Herb. No. 25.

25. Coprinus niveus (Pers.) Fr. (*Sacc.* V : 1088; *B. & B.*, 89).

On dung and heaps of rotten straw, Lohagal Village, 6-9-1953, Leg N. C. J., Myco. Herb. No. 26.

26. Ganoderma lucidum (Leyss.) Karst. (*Sacc.* VI : 157; *B. & B.*, 98)

On trunks of old trees, Mayo College Road, 6-8-1953, Leg Sunita, Myco. Herb. No. 27.

27. Scizophyllum commune Fr. (*Sacc.* V: 655; *B. & B.*, 128)

On moist dead wood at Kishangarh Road, 11-8-1953, Leg N. C. J., Myco. Herb. No. 28.

28. Podaxon carcinomalis (L.) Fr. (*Sacc.* VII : 588; *B. & B.*, 137)

On moist soil, Foysagar, 4-9-1954, Leg N. C. J., Myco. Herb. No. 29.

29. Melampsora lini (Pers.) Lév. (*Sacc.* VII : 588; *B. & B.*, 60)

On living leaves, twigs and branches of *Linum usitatissimum* L. (Linaceae), Bot. Dept. Garden, Govt. College, 12-2-1950, Leg B. T., Myco. Herb. No. 30 (Uredo- and Teleuto-stages).

30. Haplophragmiopsis ponderosum (Syd. & Butler) Thirum. (*B. & B.*, 59)

On living twigs of *Acacia leucophloea* Willd. (Leguminosae), Lohagal Road, 3-1-1952, Leg N. C. J., Myco. Herb. No. 31 (Telial stage).

31. Puccinia heterospora Berk. and Curt. (*Sacc.* VII : 695; *B. & B.*, 68)

On living leaves of *Sida* sp. (Malvaceae), Chamunda, 1-1-1953, Leg N. C. J., Myco. Herb. No. 32 (Telial stage).

32. Puccinia glumarum (Schum.) Eriks. and Henn.(*Sacc.* XVII : 380; *B. & B.*, 67)

On living leaves, culms and glumes of *Triticum* sp. (Gramineae), Tabeji Farm, 2-2-1954, Leg N. C. J., Myco. Herb. No. 33 (Uredinial and Telial stages); also on living leaves, culms and glumes of *Hordeum vulgare* (Gramineae), Kishangarh Road, 12-2-1953, Leg N. C. J., Myco. Herb. No. 34 (Uredinial and Telial stages).

33. Puccinia graminis Pers. (*Sacc.* VII : 622; *B. & B.*, 67-68)

On living leaves, culms and glumes of *Triticum* sp. (Gramineae), Tabeji Farm, 3-4-1952, Leg N. C. J., Myco. Herb. No. 35 (Uredinial and Telial stages).

34. Puccinia tumidepes Peck. (*Dalela*, 1956)

On living leaves of *Lycium europaeum* L. (Solanaceae), Tabeji Farm, 6-4-1954, Leg N. C. J., Myco. Herb. No. 36 (Uredinial and Telial stages).

35. Melampsora helioscopiae (Pers.) Wint. (*Sacc.* VII : 586; *B. & B.*, 60)

On living leaves of *Euphorbia dracunculoides* Lamk. (Euphorbiaceae), Anteer, 3-4-1954, Leg C. R., Myco. Herb. No. 37 (Uredinial and Telial stages).

36. Ravenelia ajmerensis Sanwal

On living leaves of *Acacia* sp. (Leguminosae), Adarsh Nagar, 11-11-1953 Leg B. D. T., Myco. Herb. No. 38 (Uredinial and Telial stages).

37. Ravenelia sessilis Berk. (*Sacc.* VII : 773; *B. & B.*, 77)

On living leaves and pods of *Albizzia lebbek* Benth. (Leguminosae), Civil lines, 10-11-1953, Leg N. C. J., Myco. Herb. No. 39 (Uredinial and Telial stages).

38. Ravenelia ornata Syd. (*Sacc.* XXI : 738; *B. & B.*, 77)

On living leaves of *Abrus precatorius* L. (Leguminosae) Lohagal Rade, 17-12-1954, Leg N. C. J., Myco. Herb. No. 40 (Telial stage).

39. Trochodium ajrekeri Gharse (*Gharse*, 1949)

On living leaves of *Rivea ornata* var. *Griffithii* Cl. (Convolvulaceae), Foysagar, 6-12-1953, Leg N. C. J., Myco. Herb. No. 41 (Uredinial, Telial and Aecidial stages).

40. Pericladium grewiae Pass. (*Sacc.* VII : 838 ; *Thirumulachar & Mundkur*, 1951)

On twigs, petiole and stem of *Grewia orbiculata* Rottl. (Tiliaceae), Adarsh Nagar, 15-9-1954, Leg N. C. J., Myco. Herb. No. 42.

41. Sphacelotheca sorghi (Link.) Clinton (*B. & B.* 46; *Thirumulachar & Mundkur*, 1951)

In ovaries of *Sorghum vulgare* Pers., Pushkar, 9-11-1953, Leg N. C. J., Myco. Herb. No. 43.

42. Sphacelotheca macalpineae Zundel (*Joshi*, 1955)

In inflorescence of *Andropogon controtus* L. (Gramineae), Foysagar, 10-10-1952, Leg N. C. J., Myco. Herb. No. 44 (This pathogen produces long whip-like structure like that in sugarcane).

43. Ustilago hordei (Pers.) Killerm. and Swingle (*Sacc.* IX : 263 ; *Thirmnulachar & Mundkur*, 1951)

In spikelets of *Hordeum vulgare* L. (Gramineae), Pushkar, 2-2-1953, Leg N. C. J., Myc. Herb. No. 45.

44. Ustilago tritici (Pers.) Jensen (*Sacc.* IX : 283; *B. & B.*, 5; *Thirumulachar & Mundkur*, 1951)

In spikelets of *Triticum vulgare* (Gramineae), Pushkar, 2-2-1953, Leg. N. C. J., Myco. Herb. No. 46.

45. Ustilago sparsa Underwood (*Sacc.* XIV : 416 ; *Thirumulachar* & Mundkur, 1951)

In ovaries of *Eleusine aegyptiaca* (Gramineae), Foysagar, 13-11-1953, Leg N. C. J., Myco. Herb. No. 47.

46. Ustilago cynodontis Henn. (*Sacc.* XIV : 416; *B. & B.*, 48)

In inflorescence of *Cynodon dactylon* Pers. (Gramineae), Taragarh, 17-11-1954, Leg N. C. J., Myco. Herb. No. 48.

47. Ustilago scitaminea var. sacchari-officianarum Mundkur (*Thirumulachar & Mundkur*, 1951)

In inflorescence of *Saccharum officinarum* L. (Graminae), Buddha Pushkar, 6-11-1952, Leg N. C. J., Myco. Herb. No. 49.

48. Alternaria burnsii Uppal, Patel and Kamat (*Joshi*, 1956)

On living leaves and shoots of *Cuminum cyminum* L. (Umbelliferae), Kishangarh Road, 2-2-1954, Leg N. C. J., Myco. Herb. No. 50 (This Fungus causes a serious disease of Cumin in this State).

49. Alternaria alli Nolla

On living leaves of *Allium cepa* L. (Liliaceae), Kekri, 3-3-1953, Leg J. P. A., Myco. Herb. No. 51 (This pathogen causes an epiphytotic disease on *Allium cepa* L.).

50. Cercospora personata Berk. and Curt. (*Sacc.* IV : *B. & B.*, 143)

On living leaves, petiole and stem of *Arachis hypogoea* (Leguminosae), Kekri, 2-2-1954, Leg N. C. J., Myco. Herb. No. 52.

51. Cercospora capsici Heald and Wold. (*B. & B.*, 141)

On living leaves of *Capsicum annuum* L. (Solanaceae), Pushkar, 6-9-1953, Leg N. C. J., Myco. Herb. No. 53.

52. Cercospora foeniculi Magn. (*B. & B.*, 142)

On living leaves, stem and fruits of *Foeniculum vulgare* (Umbelliferae), 6-5-1953, D. A. V. College, Ajmer, Leg N. C. J., Myco. Herb. No. 54.

53. Cephalosporium curtipes Sacc. (*Joshi*, 1954)

In seeds of *Ricinus communis* L. (Euphorbiaceae), Bot. Dept., 6-9-1953, Leg N. C. J., Myco. Herb. No. 55.

54. Fusarium moniliforme Sheldon (*Mundkur*, 1938).

Isolated from the diseased endosperm of the castor seeds, Bot. Dept., 6-9-1953, Leg N. C. J., Myco. Herb. No. 56.

55. Rhizoctonia solani Kuhn. (*Shaw et al.*, 1915)

On fruits of *Lycopersicum esculentum* L. (Solanaceae), 2-1-1955, Beer Village, Leg N.. C. J., Myco. Herb. No. 57. (On the fruits of *Lycopersicum esculentum* L. causing a serious disease in this State).

Acknowledgement

The author is grateful to Dr. M. J. Thirumalachar and Dr. S. P. Gordon for the identification of some of the Fungi and to Mr. M. B. Raizada for the identification of some of the hosts. Thanks are also due to the Principal, Government College, Ajmer and to Prof. B. Tiagi for facilities.

References

Butler, E. J. and Bisby, G. R. (1931). *The Fungi of India, I. C. A. R., Sci. Mono.*, No. 1.

Gaur, M. M. (1949). Diseases of Cumin and Fennel. *Plant. Prot. Bull.* **6**, 20–21.

Dalela, D. D. (1956). *Puccinia tumidipes* Peck on *Lycium europaeum* L. *Indian Phytopathology*, **9**, 74.

Gharse, P. S. (1949). Life-history and morphology of *Trochodium-ajrekeri* sp. nov., *J. Ind. Bot. Soc.*, **23**, 53–58.

Gupta, J. S. (1956). A new species of *Protomycopsis* on *Sesbania aculeata* Pers., *Indan. Phytopathology.*, **9**, 72–73.

Joshi, N. C. (1954). Rotting of castor seeds by *Cephalosporium curtipes* Sacc. *Sci. and Cult.*, **20**, 242.

Joshi, N. C. (1955). Occurrence of *Sphacelotheca mcalpineae* Zundel on *Heteropogon contortus* L. *Curr. Sci.*, **24**, 62–63.

Joshi, N. C. (1956). Two diseases of *Cuminum cyminum* L. from Ajmer State and methods of their control. *Sci. and Cult.*, **21**, 101–102.

Mundkur, B. B. (1938). *Fungi of India*, Supplement—I, *I. C. A. R., Sci. Mono.* No. 12.

Saccardo, P. A. (1882–1931). *Sylloge fungorum omnium hucusque cognitorum*, **1–25**.

Sanwal, B. D. (1951). Some note-worthy *Ravenelias* from India. *Sydowia*, **5**, 412–417.

Shaw, F. J. F. *et al.* (1915). The genus *Rhizoctonia. Mem. Dept. Agri.*, India., Bot. Sr. **7**, 179–184.

Thirumalachar, M. J. and Mundkur, B. B. (1951). *Ustilaginales of India. C.M.I.*, Kew Surrey, England.

(*Received for Publication 15 April 1957*)

Ind. J. mycol. Res., 2 (1&2), 71-73, 1956.

BRIEF ARTICLES

NODULATION OF *CICER ARIETINUM* L. IN FIELD-SOIL

The common gram, *Cicer arietinum* L. is cultivated throughout the plains of India as a food crop and is of high commercial value among leguminous crops. Gram plants normally produce numerous nodules of two distinct types, simple ellipsoidal nodules and complex branched ones, in fields where they are cropped every winter from October to February.

Since 1949, attempts were made to collect nodulated roots of *Cicer* from areas in and about Calcutta for isolation of *Rhizobium*. Every year *Cicer* plants were raised from different varieties of seeds in manured and unmanured experimental plots of Bethune College, Presidency College, R. G. Kar Medical College and in the New Alipore area, Calcutta, but nodules were consistently absent on the roots. The range of *p*H of the soil in the experimental plots was 5.8 to 6.8. During 1953–55, three months prior to the growing season, experimental plots had a yearly dressing of farmyard manure and of fresh soil from fields in the vicinity of Calcutta. These yearly dressings had, however, no effect on the production of nodules on roots of *Cicer* plants. This was, probably, due to the absence of suitable strains of *Rhizobium* necessary to produce nodules on these plants growing in the soil of Calcutta, paritcularly in the experimental plots. Preliminary experiments were, therefore, set up to study the effects of seed inoculation with strains of *Rhizobium* isolated from nodules of *Cicer* growing in Murshidabad district of West Bengal, India.

The plants were raised from locally collected seeds. Only well formed ripe seeds with unbroken testa were selected for the purpose. *Rhizobium* was isolated from nodules of *Cicer* plants grown in Berhampore (Murshidabad). Cultures of *Rhizobium* were maintained in *yeast-water-agar* medium (Bhaduri, 1951). Seeds were superficially sterilized with 0.04% $HgCl_2$ solution for 1 minute and subsequently washed with sterile water under aseptic condition. These were, then, kept in the suspension of bacteria in sterile tapwater made from a 2 to 3-days-old culture of *Rhizobium*. After 18 hours, the suspension was drained off and the seeds were again treated with a freshly prepared suspension of the same bacteria for 1 hour. The seeds were then aseptically sown on sterile agar slants in test-tubes ($8'' \times 1\frac{1}{2}''$). Several pots containing sterilized washed sand were also sown with similarly inoculated seeds. Plants were daily watered with culture solution just to soak the sand without overflowing the pots. Duplicate experiments were, also, set up in pots with unsterilized washed sand. In sand pots, nitrogen deficient culture solution was added as a nutrient material for the plants. To this culture solution, 2% agar was added to prepare slants in test-tubes (Bhaduri, 1951). The culture solution, however, was not added to the field plots.

Plants grown in test-tubes with added suspension of *Rhizobium* became nodulated within 15 days, while the controls remained free from nodules until the end of the experiment.

Experimental plots (6×4 ft.) in the field were sown with inoculated seeds. In the control plots the seeds were sown without inoculation. All the plants were harvested after 24 days' growth. Bacteria isolated from nodules of plants in each experiment were tested by plant passage in controlled conditions as described by Nutman (1946).

Adequate controls without inoculum were kept under identical conditions in each of the experiments. These control plants remained without nodules even after 3 months' growth (Plate I, fig. 1).Nodules produced by Berhampore strain in plots were mostly complex (Plate I, fig. 2) owing to the partial fused condition during growth of a number of elongated nodules from a few adjacent loci on a root, while those in sand pots were generally simple and spherical to ellipsoidal in shape (Plate I, fig. 3).

Table 1. *Number of nodules per plant and size of nodules*

Experiment	Number of Replicates	Number of nodules per plant	Range of mean diameter of nodules in mm.	Remarks on elongated or ellipsoidal nodules
Pot (Sterilized sand)	15	3.2±4.0	1 to 3	1 nodule, 6 mm. long
Pot (Unsterilized sand)	18	4.6±0.2	1 to 3	3 nodules, 4 mm.–5.5 mm. long
Field Plot	12	3.8±0.2	1 to 3	1 nodule, 5 mm. long

Table 1 shows that mean number of nodules of plants in these experiments were not significantly different and generally the ranges of the mean size of nodules were not variable. Sometimes, however, one or more nodules of a significantly bigger size were produced, and these were elongated or ellipsoidal in shape.

It was, further, observed that there was no residual effect even after two consecutive years of seed inoculation with the given strain of bacteria in the same plot of the field. The result of the experiments indicated that the experimental fields were devoid of the strain *Rhizobium* suitable to produce nodules on roots of *Cicer*, and that a good strain of a different locality was able to produce nodules on roots of plants grown in the soil. There is, however, reason to believe that the introduced strain of *Rhizobium* could not remain active enough to produce nodules in the succeeding year. Since *Lens esculentum* L., *Phaseolus radiatus* var. *aurea* Prain, *Phaseolus mungo* L., *Lathyrus sativus* L. or *Trigonella foenum-graecum* L. plants growing in adjacent or in between two experimental plots produced abundant nodules on their roots. it might be assumed that the micro-environment of the population of *Rhizobium* in the soil had, at least, some

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general conditions suitable for the growth of these bacteria. It has been shown that genetic factors in the legume host is of importance in the production of nodules of plants (Nutman, 1955), but *Cicer* seeds obtained from the local market and other sources were from naturally cross-pollinated plants and were evidently heterozygous in so far as the genetic factors for nodulation were involved. Such factors were not known to have any adverse effect on nodule production. The question, therefore, of establishing a strain of *Rhizobium* producing nodules on roots of *Cicer*, will depend on various factors, the chief among them may be competition between micro-organisms including different strains of *Rhizobium* and the applied strain (Thornton, 1954), presence of *Rhizobiphage* in the soil (Allen and Allen, 1950) and the secretion of antibiotic substances produced by micro-organisms of the soil including other strains of *Rhizobium* capable of attacking of applied strain of *Rhizobium* (Allen and Allen, 1950). At present we know too little to be able to assess the importance of any one or of a combination of a few or all of these factors in the establishment of any strain of bacteria in the soil specially in connexion with the legume seed inoculation.

Authors wish to acknowledge the support of Indian Council of Agricultural Research, Government of India and the Department of Agriculture, Government of West Bengal, for providing necessary finance for the continuation of these investigations. The work is in progress.

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Allen, E. K. and Allen, O. N. (1950). Biochemical and symbiotic properties of Rhizobia *Bact. Rev.*, **14**, 273–330.

Bhaduri, S. N. (1951). The influence of the numbers of *Rhizobium* supplied on subsequent nodulation of the legume host plant. *Ann. Bot., Lond.*, N. S. **15**, 209–18.

Nutman, P. S. (1946). Variation within strains of clover nodule bacteria in the size of nodule produced and in the 'effectivity' of the symbiosis. *J. Bact.*, **51**, 411–32.

Nutman, P. S. (1955). The influence of the legume in root nodule symbiosis. *Biol. Rev.*, **31**, 109–29.

Thornton, H. G. (1954). The nodule bacteria and thier host legumes : some problems that they still present. *Science Progress*, **42**, 185–204.

Ind. J. mycol. Res., **2** (1 & 2), 74–75, 1956.

STUDIES ON HETEROTHALLISM—III.

LENTINUS SUBNUDUS BERK. & *LENTINUS PRAERIGIDUS* BERK.

Investigations on the phenomenon of heterothallism in Agaricaceae has been reviewed quite extensively by previous workers like Whitehouse (1949), Quintanilha and Pinto-Lopes (1950) and Raper (1953). It appears that as far as the genus *Lentinus* is concerned very little work has been done in this direction. *Lentinus tigrinus* (Bull.) Fr., the only species of the genus, has been worked out by Quintanilha *et al* (1941) and they have reported it to be 'heterothallic' and 'tetrapolar'. It has already been mentioned in an earlier publication of Banerjee and Samadder (1957) that investigations concerning the sexual phenomena in Indian Hymenomycetes are now in progress in this laboratory. The results obtained so far on two other species of *Lentinus*, viz., *L. subnudus* and *L. praerigidus* are given below.

Polysporous cultures of both *L. subnudus* and *L. praerigidus* revealed the presence of numerous clamp-connexions while these are entirely absent in the mycelia originating from single spores. This induces the writers to think that they might be heterothallic in nature.

To determine the type of heterothallism and the number of sex groups present, twenty monosporous cultures of each fungus were made following the usual dilution method from spores obtained from a single sporophore. The sporophores were collected from a timber-yard of Calcutta while growing on logs of *Shorea robusta* Graertn. f. Of the twenty monosporous cultures of each fungus lacking clamp-connexions, twelve were paired in all possible combinations on 2.5% *malt-agar* slants and they were allowed to grow under ordinary conditions of temperature (28°–30°C.) and diffused light of the laboratory. After one month, the line of contact between the paired mycelia was carefully examined for the presence of clamp-connexions. The results of this examination are recorded in Tables 1 and 2. The sign 'plus' indicates the presence of clamp-connexions and the sign 'minus' indicates their absence.

From Tables 1 and 2 it is evident that both the species are 'heterothallic' and sexually 'bipolar'. In both the cases, spores obtained from a single sporophore fall into two groups and they can only form clamp-connexions when members of one group unite with the members of the opposite group. It may be assumed that the sexual compatibility of these two species is governed by one pair of factors, Aa, located on the same locus of different chromosomes and behaves in a similar manner as do the heterothallic species of Mucorales.

Incidentally, three principal types of visually recognisable interactions between monosporous mycelia of both *L. subnudus* and *L. praerigidus* have been recorded which are as follows :

Neutral.—The two monosporous cultures meet freely to form a homogeneous mat so that the identity of the interacting mycelia is lost. There is no line of demarcation between the two uniting mycelia and no secondary mycelium with clamp-connexions is produced.

Compatible.—The two monosporous mycelia meet and intermingle in such a way that the identity of the interacting mycelia is completely obliterated. A homogeneous mat with typical secondary mycelium having clamp-connexions is also produced.

Table. 1. *Pairings of 12 monoporous mycelia derived from a single sporophore of* Lentinus subnudus.

	1	2	4	5	7	11	12	3	6	8	9	10
1		−	−	−	−	−	−	+	+	+	+	+
2	−		−	−	−	−	−	+	+	+	+	+
4	−	−		−	−	−	−	+	+	+	+	+
5	−	−	−		−	−	−	+	+	+	+	+
7	−	−	−	−		−	−	+	+	+	+	+
6	−	−	−	−	−		−	+	+	+	+	+
12	+	−	−	−	−	−		+	+	+	+	+
3	+	+	+	+	+	+	+		−	−	−	−
6	+	+	+	+	+	+	+	−		−	−	−
8	+	+	+	+	+	+	+	−	−		−	−
9	+	+	+	+	+	+	+	−	−	−		−
10	+	+	+	+	+	+	+	−	−	−	−	

Table 2. *Pairings of 12 monoporous mycelia derived from a single sporophore of* Lentinus praerigidus.

	2	3	4	5	6	12	1	7	8	9	10	11
2		−	−	−	−	−	+	+	+	+	+	+
3	−		−	−	−	−	+	+	+	+	+	+
4	−	−		−	−	−	+	+	+	+	+	+
5	−	−	−		−	−	+	+	+	+	+	+
6	−	−	−	−		−	+	+	+	+	+	+
12	−	−	−	−	−		+	+	+	+	+	+
1	+	+	+	+	+	+		−	−	−	−	−
7	+	+	+	+	+	+	−		−	−	−	−
8	+	+	+	+	+	+	−	−		−	−	−
9	+	+	+	+	+	+	−	−	−		−	−
10	+	+	+	+	+	+	−	−	−	−		−
11	+	+	+	+	+	+	−	−	−	−	−	

Antagonistic.—The two monosporous mycelia meet but form a line of demarcation between them. Typical secondary mycelium with clamp-connexions is not produced.

Of the two species, *Lentinus subnudus* exhibits only the first two types of reactions, viz., neutral and compatible and hence behaves normally like a typical bipolar species. *Lentinus praerigidus*, on the other hand, presents all the three types of reactions, viz., neutral, compatible and antagonistic. In spite of the last named reaction, which is characteristically noticeable in tetrapolar species, *Lentinus praeridigus* is considered as a 'bipolar' one, based on the presence or absence of clamp-connexions in the paired cultures.

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Banerjee, S. N. and Samadder, K. R. (1957). Studies on Heterothallism—II. *Polystictus xanthopus* Fr. *Science and Culture*, **22**, 578–579.
Quintanilha, A., Quintanilha, L. et Vassermanis, A. (1941). La condiute sexuelle *et la* Systematique des Hymenomycetes. *Revue de Mycologie*, **66**, 3–48.
Quintanilha, A. and Pinto-Lopes, J. (1950). La conduite des espèces d'Hymenomycètes. *Boletim da Sociedade Broteriana*, **24**, 115–290.
Raper, J. R. (1953). Tetrapolar sexuality. *Quart. Rev. Biol.*, **29**, 233–259.
Whitehouse, H. L. K. (1949). Heterothallism and Sex in Fungi. *Biol. Rev.*, **24**, 411–447.

Ind. J. mycol. Res., **2** (1 & 2), 76–77, 1956.

HOST-RANGE AND DISTRIBUTION OF *DAEDALEA FLAVIDA* LÉV.

Daedalea flavida Lév. is one of the common wood-rotting tropical polypores in India that attack our structural and standing timbers. Its large, conspicuous, applanate and corky brackets with characteristic labyrinthiform pore-mouths appear in great abundance on logs, posts and dead branches of forest trees during wet months of the year. The host list that follows has been compiled from the available reports of Banerjee (1947), "The list of common names of Indian Plant Diseases" published by the Indian Council of Agricultural Research (1950) and also from the list of collections in the "Polyporaceae Herbarium" of Dr. S. R. Bose at R. G. Kar Medical College, Calcutta. This list contains all the host-species so far known to the authors and cannot be claimed to be complete in itself, pending extensive collections covering wide areas of India and abroad. The names of the hosts are given below.

Acer caesium Wall., *Anogeissus latifolia* Wall., *Acacia arabica* Willd., *Alnus nepalensis* D. Don., *Areca catechu* L.. *Bambusa sp.*. *Borassus flabellifer* L., *Bombax malabaricum* DC., *Cocos nucifera* L.. *Casuarina equisetifolia* L.. *Dalbergia sisso* Roxb., *Excoecaria agalocha* L., *Ficus bengalensis* L.. *Ficus religiosa* L., *Hymenodictyon excelsum* Wall., *Heritiera minor* Roxb., *Lagerstroemia lanceolata* Wall., *Mangifera indica* L.. *Melaleuca leucadendron* L., *Phoenix sylvestris* Roxb., *Shorea robusta* Gaertn. f.. *Swietenia mahagoni* L., *Sonneratia apetala* Ham., *Terminalia paniculata* W. and A., *T. tomentosa* W. and A., *T. belerica* Roxb., *Tectona grandis* L.. *Tamarindus indica* L.. *Tetrameles nudiflora* R. Br.

To this list should be added *Ficus infectoria* Roxb.. *Grewia asiatica* L.. *Mimusops elengi* L. and *Terminalia catappa* L., which have been recorded by the writers during field-collections.

From the above list it is evident that *Daedalea flavida* Lév. mostly grows on a wide range of hard wood species. It also occurs on a number of monocotyledons but no coniferous host has yet been recorded. The wide range of host-wood species includes some of the valuable timber yielding plants and others are of various economic importance.

So far very little is known about the geographical distribution of *Daedalea flavida*. Its occurrence in Australia, Borneo, Java, Sumatra and India (Sacc., 1888; Butler and Bisby, 1931) and also in East Pakistan. Burma, Andaman Islands, Malaya and the Philippines* indicates its distribution in the tropics. It has not yet been recorded from the temperate regions of the world. It occurs throughout West Bengal from the Sunderbans in the south to Jalpaiguri and Darjeeling districts in the north. In other

* From the records in the 'Polyporaceae Herbarium' of Dr. S. R. Bose

parts of India its distribution is probably wider but the records appear to be very meagre. As the records go, it has, so far been collected from

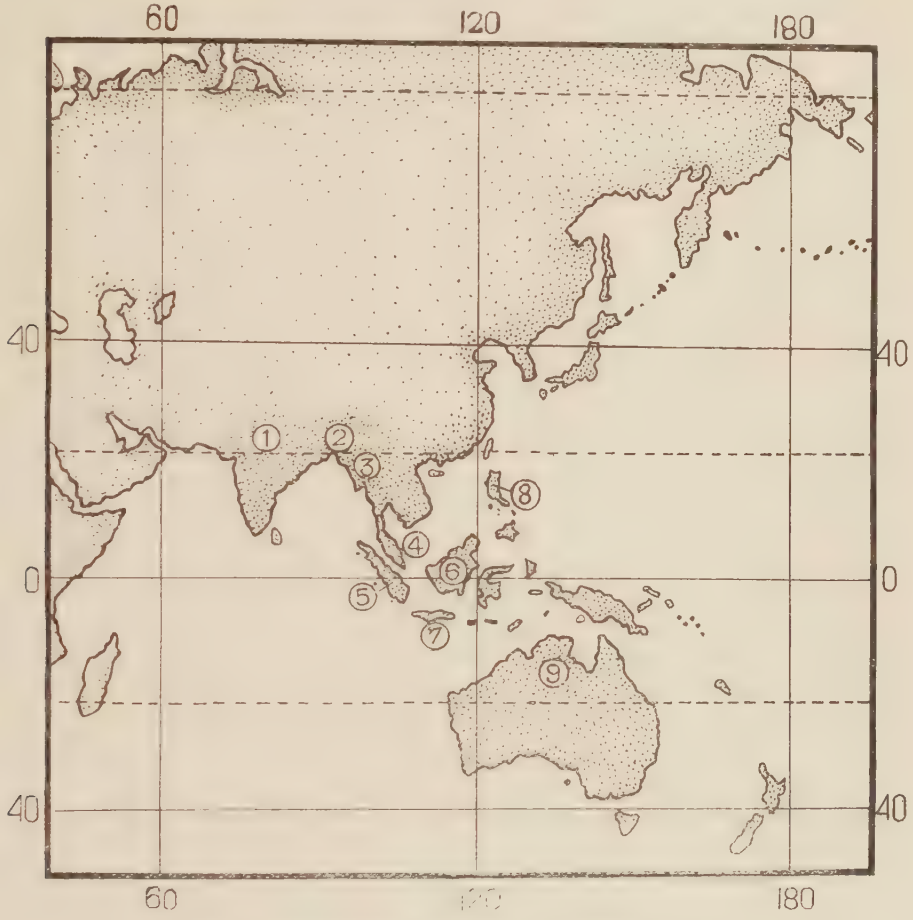

Fig. 1. *Map showing distribution of* Daedalea flavida

1, India ; 2, East Pakistan ; 3, Burma ; 4, Malay ; 5, Sumatra ; 6, Borneo ; 7, Java ; 8, The Philippines and 9, Australia.

Tripura, Assam, Orissa, Bihar, Uttar Pradesh, Madhya Pradesh, Bombay and Madras only (Fig. 1).

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Banerjee, S. N. (1947). Funguous flora of Calcutta and Suburbs. *Bull. bot. Soc. Beng.*, **1**, 37–54.

Butler, E. J. and Bisby, G. R. (1931). *The Fungi of India*, Sci. Mono. **1**, Indian Coun. Agric. Res., 91.

Saccardo, P. A. (1888). *Sylloge fungorum omnium hucusque congnitorum*, **6**.

Ind. J. mycol. Res., **2** (1 & 2), 78–79, 1956.

EFFECT OF GLUCOSE CONCENTRATION ON GROWTH OF CERTAIN BASIDIOMYCETES

Of all the carbohydrates, glucose is more utilized by the fungi than any other sugar and it is nearly a universal source of carbon. Hawker (1939) described the effects of a range of concentrations of glucose and some other sugars on growth and fruiting of *Melanospora destruens* Shear. He showed that mycelial growth increased with increase in the concentration of glucose up to a point above which a gradual decline in growth was noticed. Similar results were also obtained by Brown (1925) with *Fusarium fructigenum* and by Hawker and Chaudhuri (1946) with several species of Ascomycetes. This paper presents the results obtained from a study on the effect of different concentrations of glucose on the amount of growth of three species of Polyporaceae, viz., *Polystictus xanthopus* Fr., *Polystictus sanguineus* (L.) Mey., and *Trametes persoonii* Fr., all of which are responsible for the decay of wood.

The cultures used for this investigation were obtained by tissue-culture method from fresh sporophores. *Glucose-asparagine solution* was selected as the basal medium for this investigation. Its composition per litre of distilled water was KH_2PO_4, 1.5 gm.; $MgSO_4$ $7H_2O$, 0.5 gm.; Asparagine, 2 gm.; and trace elements in p.p.m.—Boron, 0.005; Copper, 0.02; Iron, 0.1 and Manganese, 0.01. Glucose was added in varying amounts to this medium to obtain 1.0, 2.0, 3.0, 4.0 and 5.0 per cent conncentrations.

The *p*H of the medium was adjusted to 6.0 before autoclaving. 15 m.l. of the medium was distributed in each of the Erlenmeyer flasks of 100 m.l. capacity. Sterilization of the medium was done at 15 lb. pressure for 15 minutes.

Replicates of three flasks were employed and results, reported here, were based on the average of three cultures. The inoculum was taken from the advancing region of the actively growing colonies on *potato-dextrose-agar*. Thin and uniform discs (5 mm. in diameter) cut out from the plate were used as inocula. The cultures were incubated at 25°C. for a period of 14 days during which they were aerated daily by shaking for $\frac{1}{2}$ minute. Growth was determined after harvesting. The mycelium was obtained after filtering the medium through a finely woven cloth and any trace of the medium was removed by washing it several times with hot water. The mycelium was then carefully transferred to a weighing bottle and dried to a constant weight at 90°C. The *p*H of the medium at the time of harvest was about 5.0 in all cases. The results are recorded in Table 1.

From Table 1, it will be evident that the three test fungi are essentially similar in nature in their reactions to different concentrations of glucose. Growth, in all the three species, increased with the use of more glucose up to a certain concentration, varying with species, above which it declined rapidly in case of *P. xanthopus* and *T. persoonii* and gradually in case of *P. sanguineus*. In *P. sanguineus* and *T. persoonii* optimum growth was

obtained at the same concentration (3%). Of these two species, *T. persoonii* had a well defined, narrow, optimum value at about 3% but *P. sanguineus* showed a broad range of tolerance, with nearly maximum growth all the

Table 1. *Mycelium (in mg.) of three fungi produced on glucose-asparagine medium.*

Fungus	Concentrations of glucose in the medium				
	1%	2%	3%	4%	5%
Polystictus xanthopus	55	74	76	83	40
Polystictus sanguineus	40	94	102	92	69
Trametes persoonii	59	83	129	80	48

way from a concentration of 2% to 4%. With *P. xanthopus* optimum was at a slightly higher concentration (4%). In the latter species a near-maximum growth was also recorded throughout the concentration range from 2% to 4%. From the results it will appear that the two species of the genus *Polystictus* viz., *P. xanthopus* and *P. sanguineus* have the same range of tolerance (2% to 4%) but they differ somewhat in their optimal concentration of glucose. Thus, it will be seen that earlier observations by Brown (1925) Hawker (1939) and Hawker and Chaudhuri (1946) are in agreement with the results of the presnt investigation.

This investigation has only attempted to give an approximate idea of the optimum concentration of glucose necessary for the rapid growth of three species of Basidiomycetes. An accurate determination of the optimum concentration, however, requires the use of a larger number nutrient solutions differing in their glucose concentration by much smaller values, but that has not been possible in the present case.

The author is grateful to Dr. S. N. Banerjee who kindly supplied fresh sporophores for tissue-culture in this work. Thanks are, also, due to my colleagues, Dr. J. C. Saha, Head of the Dept. of Botany-cum-Biology and Sri D. R. Chatterjee of the Dept. of Chemistry for their co-operation.

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Brown, W. (1925). Studies in the genus *Fusarium* II. An analysis of factors which determine the growth-forms of certain strains. *Ann. Bot., Lond.*, **39**, 373–408.

Hawker, L. E. (1939). The influence of various sources of carbon on the formation of perithecia by *Melanospora destruens* Shear in the presence of accessory growth factors, *Ann. Bot., Lond.*, N. S., **3**, 455–468.

Hawker, L. E. and Chaudhuri, S. D. (1946). Growth and fruiting of certain Ascomcetous fungi as influenced by the nature and concentration of carbyhydrate in the medium. *Ann. Bot., Lond.*, N.S., **10**, 185–194.

Ind. J. mycol. Res., **2** (1&2), 80-82, 1956.

FORMATION OF SPOROPHORES OF *GANODERMA LUCIDUM* (LEYSS.) KARST. AND *GANODERMA APPLANATUM* (PERS.) PAT. IN CULTURE

Production of typical sporophores of wood-rotting fungi in culture still remains a great problem, as no single and standard method has yet been devised that can be applied in all cases. This difficulty was also experienced by the writers for obtaining sporophores of both *Ganoderma lucidum* and *Ganoderma applanatum* which are found so commonly in Bengal. There are, however, some records that attempts were made to obtain typical sporophores of *Ganoderma* in culture. Long and Harsch (1918) claimed to have produced typical pilei of *G. lucidum* on agar medium. Bose (1929) succeeded in getting 'porous area' of *G. lucidum* on *malt-agar*. Badcock (1943), by following his own method, was able to produce typical pileus of *Ganoderma resinaceous* (=*G. lucidum*). Venkatarayan (1936), following White (1919), used *Areca* stems as the medium and obtained some 'hemispherical lumps' of *G. lucidum* which shed normal basidiospores.

At first trials were made with various agar media such as 2.5% *malt-agar* *potato-dextrose-agar* and *Czapeck's synthetic-agar* but none of the fungi produced typical fruit-bodies in any of them, even under different conditions of light, temperature and humidity of the laboratory. Occasionally, however, loose porous area containing typical spores of *G. lucidum* were obtained on 2.5% *malt-agar* and *potato-dextrose-agar*. Badcock's (1941) saw-dust medium and the various devices described by him for obtaining an atmosphere of relatively high humidity favourable for the development of sporophores, were then tried, without any success.

All attempts to produce typical sporophores of *G. lucidum* and *G. applanatum* on agar and Badcock's media being a failure, an experiment was started to find out whether the secondary mycelia of the two fungi could be induced to fructify on wood-blocks of their selective host. In Bengal, common host of *G. lucidum* is *Casuarina equisetifolia* and that of *G. applanatum* is *Mangifera indica*. Sapwood of the host-species was selected for the purpose and the experimental procedure employed for each was as follows : sterile and thoroughly water-soaked sapwood-blocks ($5 \times 3 \times 2$ c.m.) were introduced into sterilized Kolle flasks and placed over 7-days-old secondary mycelium of the fungus on 2.5% *malt-agar* slants already made available for the purpose. In each flask five such wood-blocks were introduced and they were arranged side by side over the surface of the growing mycelium. These were then kept in complete darkness and under ordinary temperature (30°—33°C.) of the laboratory.

Within ten days the wood-blocks (*Casuarina equisetifolia*) were vigorously attacked on all sides by a luxuriant growth of the mycelium of *G. lucidum* and in about a month they became completely covered by a thick and tough mat. After two months, a few mycetial 'knots' were developed here and there on the surface of the mat. Some of these 'knots' rapidly increased in size, and on the 68th day, looked like small 'knobs'. These

knobs, when first formed, were round and perfectly white in colour. In next 3 or 4 days, they became slightly elongated (Pl. I, fig. A), showed a tendency of curving towards the neck of the flask (Pl. I, fig. B) and the upper part of the fruit-body became dull brown in colour. Within a couple of days, a porous area developed at the tip of each knob and this area became surrounded by a sterile rim-like white margin (Pl. I, fig. C). Within the flask, these knob-like fruit-bodies did not grow further. At this stage, the entire solid mat containing the wood-blocks and the fruit-bodies were taken out of the flask carefully and was placed on a moist cotton pad in a Petri-dish. The Petri-dish was then kept under a tap which was adjusted in such a way that only the knobs could get a constant supply of water in the form of small drops. As a result, the knobs soon increased in size, their apical positions expanded horizontally and became flattened, forming typical pilei within a month. In this way, other typical fructifications, each with a distinctly laccate upper surface and a lower fertile hymenial surface, were obtained (Pl. I, fig. D). The average dimension of the fruit-bodies was $5 \times 2 \times 1$ cm.

In case of *G. applanatum*, all the wood-blocks (*Mangifera indica*) became covered by thin, white, mycelial growth within a week. This aerial mycelium soon began to condense, and within a fortnight one or more white and slightly flattened structures appeared at the edge of the wood-blocks in some of the flasks (Pl. I, fig. E). The apical portion of such a rudimentary fruit-body soon began to expand laterally towards the neck of the flask forming a bracket-shaped pileus with characteristic upper surface (Pl. I, fig. F). In some cases, it formed an irregular growth before expansion (Pl. I, fig. F), but in no case typical hymenial surface could develop. This was due to the fact that during the growth of the fruit-body, its lower surface became adpressed to the inner wall of the neck of the flask in such a way as to check the formation of pore-tubes. To obviate this difficulty, some of the blocks containing the knobs were carefully taken out of the flask and placed on moist cotton in Petri-dishes. To ensure regular supply of water to the rudimentary sporophores, moist cotton pads were placed over the wood-blocks and in contact with the developing sporophores. On the 15th day after exposure, the knobs became dull brown in colour and were found to increase in length. Their whitish apices began to flatten and formed small bracket-shaped pilei. Minute shallow pores appeared on the lower side of these flattened portions and within a couple of days these became prominent. Gradually, the entire fruit-body began to increase in size. Its apical portion became flat and somewhat curved, its porous area became entirely localized to its lower surface, and finally a bracket-shaped fruit body was formed (Pl. I, figs. G, H). The average dimension of the fruit bodies was $3.5 \times 2 \times 1$ cm.

Spore-deposits were taken from these sporophores of both the fungi on 2.5% *malt-agar*. Spores were discharged profusely and they were all found to be secondary in origin because sections through the hymenial surface showed total absence of basidia within the pore-tubes and these were found to be borne directly by the hyaline, thin-walled hyphae that projected into the pore-cavities.

From the aforesaid account, it become apparent that for the production of a typical fruit-body of *Ganoderma*, the following conditions should be satisfied, viz., (*i*) sufficient supply of food and moisture, (*ii*) proper aeration, (*iii*) suitable temperature and (*iv*) probably, proper light. In the absence of proper supply of any of these factors the fungus does not produce normal sporophores under cultural conditions. These experiments further reveal another important fact that, in an attempt to fructify a wood-rotting fungus, the relationship existing between the fungus and its host wood and also the environmental conditions under which the fungus grows in Nature should be given due consideration, as pointed out by Banerjee and Sinha (1955).

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Badcock, E. C. (1941). New Methods for the Cultivation of Wood-rotting Fungi. *Trans. Brit. mycol. Soc.*, **25**, 200–205.

Badcock, E. C. (1943). Methods for obtaining Fructifications of Wood-rotting Fungi in Culture. *Trans. Brit. mycol. Soc.*, **26**, 127–132.

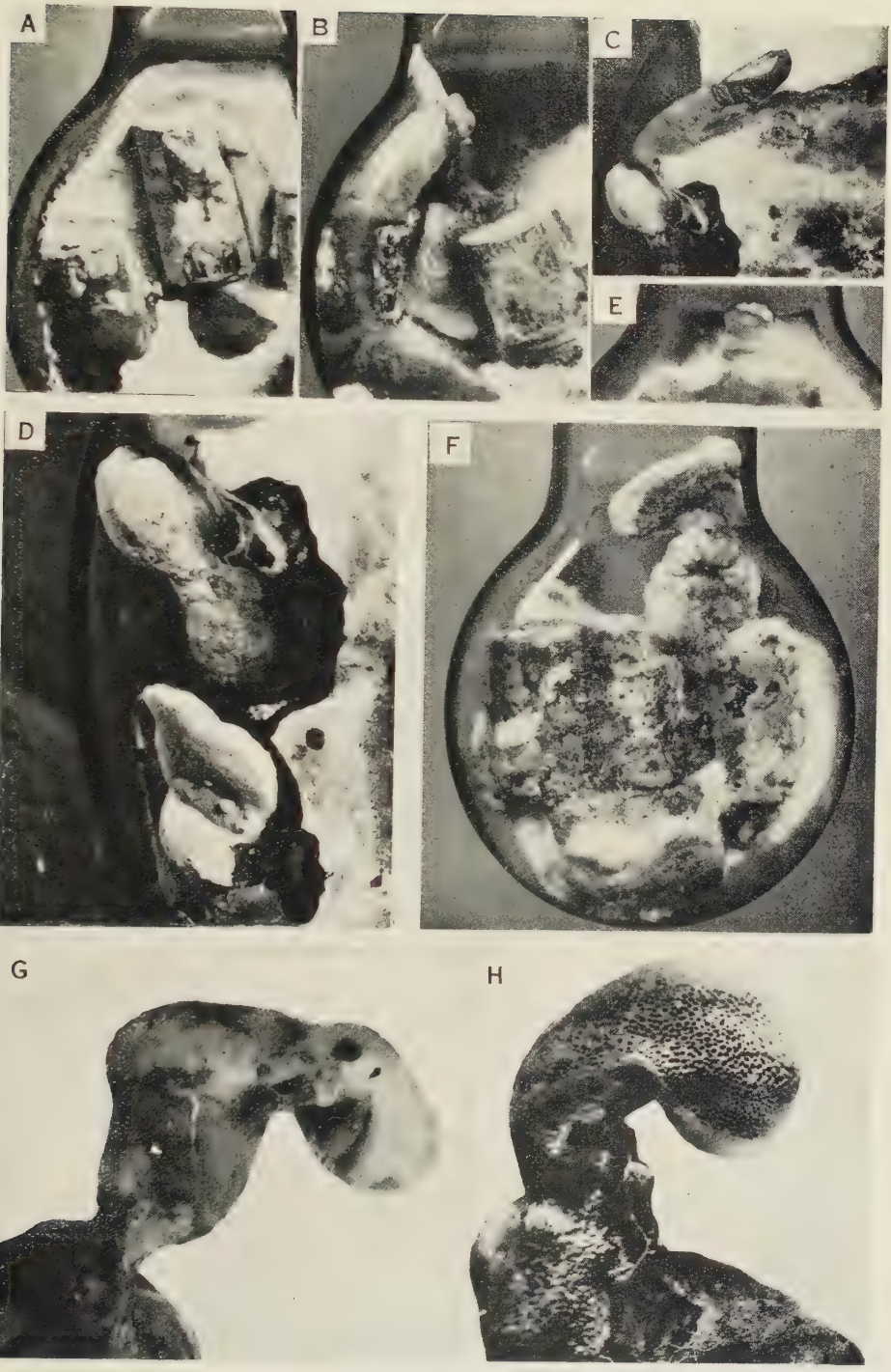
Banerjee, S. N. and Sinha, A. K. (1955). A simple method for producing typical sporophores of *Polystictus sangiuneus* (L). Mey. *Science and Culture*, **20**, 612–613.

Bose, S. R. (1929). Artificial Culture of *Ganoderma lucidum* Leyss. from spore to spore. *Bot. Gaz.*, **87**, 665–667..

Long, W. H., and Harsch, R. M. (1918). Pure cultures of Wood-rotting Fungi on artificial media. *J. agric. Res.*, **12**, 33–82.

Venkatarayan, S. V. (1936). The Biology of *Ganoderma lucidum* on *Areca* and Cocoanut Plams. *Phytopathology.*, **26**, 153–175.

White, J. H. (1919). On the Biology of *Fomes applanatus* (Pers). Wallr. *Trans. Roy. Cand. Inst., Toronto.*, **12**, 133–174.



Fruit-body formation in *Ganoderma* S. N. Banerjee & Anjali Sarkar

Ind. J. mycol. Res., **2** (1 & 2), 83–86, 1956.

WILT OF EGG-PLANT (*SOLANUM MELONGENA* L.)

Egg-plant (*Solanum melongena* L.), an important vegetable crop in the State of West Bengal, is grown in the months of June to November. The plant suffers from a wilt disease which appears in small groups of plants in the field in an irregular fashion and progresses along the rows. Plants are attacked in all stages of growth and die. The root system of the wilted plants is poorly developed and discoloured. The secondary and the tertiary roots are badly damaged and the taproot is also affected. The xylem vessels of the roots have been found to be filled up with fungal hyphae.

Records of wilt of egg-plant are very few in India. In recent years, Patel *et al* (1949) reported wilt of egg-plant caused by a species of *Verticillium*. Butler and Bishby (1931) report the occurrence of dry root-rot caused by *Macrophomina phaseoli* (Maubl.) Ashby. As this disease causes extensive damage in West Bengal, investigation was taken up to find out the cause of the disease.

Isolation and identification of the causal organism.—Small bits of affected roots, 0.5 c.m. in length, were surface sterilized with 0.1% Mercuric Chloride solution for one minute and transferred to sterile Petri dishes containing 2% plain agar. After 3-4 days' incubation at 26°—28°C. when good mycelial growth was obtained bits of mycelium were transferred to *potato-dextrose-agar* medium. In this way several isolations were made from the samples collected from different localities. The isolates sporulated within a few days and monoconidial cultures were made for the purpose of study. The isolates after careful study were found to be the same fungus in all cases. It was identified to be a species of *Fusarium*.

When superficially sterilized affected roots were incubated at 30°C. on sterile glass slides in a humid chamber, abundant mycelial growth appeared on their surface. Microscopic examination of the fungal growth also showed the presence of the same species of *Fusarium* in all cases. After careful study the fungus was identified as *Fusarium solani* (Mart.) App. and Wr. Sensu Snyder and Hansen.

Pathogenecity tests.—A number of experiments were conducted to test the pathogenecity of the fungus on egg-plant. Sutton's "Long Purple" variety was used in all the cases.

Experiment 1. Eleven sterile culture-tubes (8″ × 1″), each containing 2″ layer of sand and 10 c.cm. of nutrient solution (Sachs), were inoculated with the conidial suspension from a 21-days-old culture of the fungus on *potato-dextrose-agar* medium, while four others were kept as controls. Four seeds, surface sterilized with 0.1% mercuric chloride, were then introduced into each culture-tube and the sand was regularly moistened with sterile nutrient solution.

Experiment 2. Seven jars (5″ × 4″), each containing sterilized (with formalin) garden soil, were inoculated in the usual way with the fungal culture grown in *sand-maize-medium* for three weeks. Two jars were left as controls. After 7 days of inoculation in each of the inoculated jars as well in the controls, 30 surface sterilized seeds were sown. The soil

was kept moist throughout the experiment. The results of experiments 1 and 2 are given below.

		Number of seedlings coming up after 12 days of sowing	Number of seedlings wilted after		Total number of seedlings wilted
			7 days of emergence	15 days of emergence	
Expt. 1	Inoculated	22	9	12	21
	Control	14	nil	nil	nil
Expt. 2	Inoculated	60	35	14	49
	Control	22	nil	nil	nil

Experiment 3. A similar experiment was conducted in which instead of putting seeds, 2 to 3-weeks-old seedlings, raised in sterilized soil from the surface sterilized seeds, were transplanted in each of the five inoculated jars as well as in the two controls. Out of the ten seedlings in the inoculated jars one showed signs of wilting within 15 days of transplantation, whereas within a period of four weeks, altogether 8 seedlings wilted.

Experiment 4. Ten sterile conical flasks, each containing 100 c.cm. of Sachs' nutrient solution were inoculated with the 3-weeks-old culture of the pathogen and incubated, and 4 were kept as controls. One 3-weeks-old seedling was put in each flask after 5 days and kept in position with the help of sterilized cotton plug. All the flasks were covered with black paper. Nutrient solution was added in each flask periodically. Within a week the seedlings in the inoculated flasks showed signs of gradual yellowing of leaves and wilting, whereas the plants in the control sets did not show any such symptoms. Wilted seedlings showed poor development of the root system. The growth of the tap root was arrested and the production of secondary and tertiary roots affected. Microscopic examination of the roots showed discolouration of the vascular zones.

The same fungus was, however, reisolated from all the diseased plants of different experiments. From these experiments it is evident that *Fusarium solani* (Mart.) App. and Wr. Sensu Synder et Hansen is pathogenic to egg-plant, and it can affect the root system.

MORPHOLOGY OF THE FUNGUS.—Aerial mycelium scanty to moderate, colour white to pinkish or various shades of Pink to Pinkish cinnamon. Sporodochia present. Conidia either in sporodochia or in loosely arranged heads; both microconidia and macroconidia present. Conidia hyaline, in mass Pale Ochraceous to Ochraceous buff in colour. Microconidia oval, one-celled. Macroconidia thick-walled, almost straight but with both ends bent or curved, more or less of uniform diameter, 1-3 septate, rarely 4-septate, majority 3-septate. Chlamydospores few to abundant, warty, single or in chains, oval and intercalary (8-19.2×6.4-11.2μ) or spherical and terminal (6.4-11.2μ).

MEASUREMENTS OF CONIDIA ON DIFFERENT MEDIA

Potato-dextrose agar (containing 2% dextrose) : culture 21-days-old

CONIDIA

0-septate (18 per cent.) 11·7×2.1 (8·16×1.6—3.2)μ
1-septate (29 per cent.) 16.7×3.4 (12.8—24×3.2—4)μ
2-septate (3 per cent.) 26.4×3.8 (22.4—30.4×3.2—4.2))μ
3-septate (50 per cent.) 34×4.3 (27.2—40×3.2—4.8)μ

Potato-dextrose agar (containing 5% dextrose) : culture 21-days-old

CONIDIA

0-septate (13 per cent.) 10.8×1.9 (8—12.8×1.6—3.2)μ
1-septate (26 per cent·) 18.3×3.3 (12.8—25.6×2.4—4)μ
2-septate (13 per cent.) 25.5×3.8 (20.8—27.2×3.2—4)μ
3-septate (48 per cent.) 31.3×3.8 (25.6—36.8×3.2—4)μ

Maize agar : culture 21-days-old

CONIDIA

0-septate (7 per cent.) 11.5×2.6 (8—16×1.6—4)μ
1-septate (17 per cent.) 17.3×3.4 (11.2—20.8×3.2—4)μ
2-septate (14 per cent.) 28.5×3.4 (25.6—30.4×3.2—4)μ
3-septate (62 per cent.) 31.5×3.8 (25.6—38.4×3.2—4)μ

Brown's synthetic agar : culture 21-days-old

CONIDIA.

0-septate (29 per cent.) 9.7×1.8 (6.4—12.8×1.6—2.4)μ
1-septate (13 per cent.) 15.4×2.2 (8—24×1.6—2.4)μ
2-septate (8 per cent.) 22.4×3.0 (19.2—24×2.4—3.2)μ
3-septate (47 per cent.) 35.8×3.6 (22.4—40×3.2—4.8)μ
4-septate (3 per cent.) 36.8×4.2 (35.2—40×3.2—4.8)μ

Steamed Rice : culture 21-days-old

CONIDIA

0-septate (44 per cent.) 10.2×2.3 (6.4—14.4×1.6—3.2)μ
1-septate (47 per cent.) 17×3.1 (12.8—22.4×2.4—4)μ
2-septate (8 per cent.) 23×4 (17.6—25.6×4)μ
3-septate (1 per cent.) 28.8×4 (25—30×4)μ

Potato plug : culture 21-days-old

CONIDIA

0-septate (18 per cent.) 9.6×1.8 (6.4—16×1.6—2.4)μ
1-septate (28 per cent.) 20×2.9 (11.2—24×2.4—3.2)μ
2-septate (13 per cent) 23.4×3.4 (17.6—28.8×2.4—4)μ
3-septate (41 per cent.) 30.3×3.4 (24—38.4×3.2—4)μ

AVERAGE OF THE ABOVE MEASUREMENTS

CONIDIA

0-septate (21 per cent.) 10.7×2.1 (9.6—11.7×1.8—2.6)μ
1-septate (27 per cent.) 17.5×3 (15.4—10×2.2—3.4)μ
2-septate (10 per cent.) 25×3.6 (22.4—28.5×3—4)μ
3-septate (41.5 per cent.) 33.6×3.8 (28.8—35.8×3.4—4.3)μ
4-septate (0.5 per cent) 36.8×4.2 (35.2—40×3.2—4.8)μ

CULTURAL CHARACTERS.—From the taxonomic point of view, cultural studies of *Fusaria* are by far the most important. Accordingly, the fungus

was grown on different solid media and sterilized plant tissues to study the nature of growth and pigmentation. Study on agar media was made in Petri dishes (4″ diameter) into each of which 20 c.cm. of medium was poured. Inoculations in all the cases were made from 2-weeks-old culture on *potato-dextrose-agar*. All the cultures were kept at a temperature which varied from 26 C. to 28 C. They were exposed to diffused sunlight during the day and to the light of 60 watt electric bulb at night. The colour of mycelium has been expressed according to Ridgway (1912). The details of the cultural characteristics are presented in Table 1.

Table 1. *Cultural characters of the fungus on different media*

Media	Habit of growth	Colour
Potato-dextrose-agar (2% Dextrose)	Mycelial growth poor to moderate; hyphae short, coarse; growth downy, after 10 days depressed and matted; in about 20 days innumerable slimy spore masses appear; spore masses scattered irregularly.	White
Potato-dextrose-agar (5% Dextrose)	Mycelial growth moderate; hyphae short, coarse; growth downy, may show tendencies of being floccose occasionally; after 10-12 days mycelial growth depressed and matted; numerous spore masses appear; spore masses smaller and less in number than those in 2% *potato-dextrose-agar*.	Pinkish white
Maize-agar	Mycelial growth moderate; hyphae short, coarse; growth felty to downy; soon becoming appressed and matted; in 20 days numerous spore masses appear scattered throughout the surface.	White touched with pinkish hue
Brown's synthetic-agar	Mycelial growth scanty; hyphae short,, coarse; growth appressed, becoming matted in 10-12 days; abundant spore masses appear in three weeks' time; spore masses shiny in appearance and scattered.	White touched with very faint pinkish shade
Steamed rice	Mycelial growth moderate; hyphae coarse and short; spore masses not observed.	Pale Pinkish cinnamon
Potato plug	Mycelial growth thick; growth felty in the beginning, later on becoming appressed; numerous spore masses appear and scattered irregularly.	White with pinkish shade with pale Ochraceous buff spore pustules

Our sincere thanks are due to Dr. W. L. Gordon, Plant Pathologist, Dominion Plant Pathological Laboratory, Winnipeg, Mannitoba, Canada for the identification of the fungus.

State Agricultural Research Institute,
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June 10, 1957.

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Butler, E. J. & Bisby, G. R. (1931). *Fungi of India*. Sci. Mono. 1. Indian Coun. Agric. Res.
Patel, M. K., Qureshi, I. M. and Bhide, V. P. (1949). First Record of *Verticillium* wilt in India. *Indian Phytopathology*, **2**, 244-245.
Ridgway, (1912). *Color Standard and Color Nomenclature*, Washington, D.C.

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Published by Sri Madhusudan Chakraborty for
Mycological Society, Mycological Laboratory, Department of Botany,
University College of Science, 35, Ballygunge Circular Road, Calcutta-19,
and Printed by Sri Kalipada Mukherjee at Eka Press, 204/1, B. T. Road, Calcutta-35.

COVER & ART PLATES ENGRAVED & PRINTED BY REPRODUCTION SYNDICATE, CALCUTTA 6